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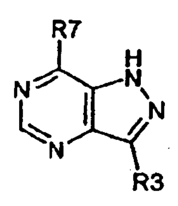
(54) Pyrazolo[4,3-D]pyrimidines, process for their preparation and methods of use

(57) The invention relates to 3-, 7-disubstituted pyrazolo[4,3-d]pyrimidines represented by the general formula I



I

and pharmaceutically acceptable salts thereof, wherein
R3 is selected from the group consisting of alkyl, cycloalkyl,, cycloalkyl alkyl, cycloheteroalkyl alkyl, cycloheteroalkyl, aryl, heterocycle, heteroaryl, arylalkyl, heteroarylalkyl, and heteroalkyl, wherein each of the groups may optionally be substituted.
R7 is selected from the group consisting of halogen, hydroxyl, hydroxylamino, amino, carboxyl, cyano, nitro, amido, sulfo, sulfamido, carbamino, unsubstituted or substituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted arylalkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroarylakyl, substituted or unsubstituted cycloalkyl alkyl, substituted or unsubstituted cycloheteroalkyl alkyl;
R7'-X- wherein X is an -NH-, -N(alkyl)-, -O- or -S- moiety and
R7' is selected from the group consisting ofH, alkyl, cycloalkyl, aryl, alkylcycloalkyl, arylalkyl, heterocycle, heterocyclealkyl, substituted alkyl, substituted cycloalkyl, substituted aryl, substituted arylalkyl, substituted heterocycle, substituted heteroaryl, substituted heteroarylalkyl, substituted heteroalkyl, substituted cycloalkyl alkyl and substituted cycloheteroalkyl alkyl,
wherein the groups are preferably substituted by more than one halogen, hydroxyl, amino, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, carbamino, alkyl, alkoxy, and substituted alkyl group

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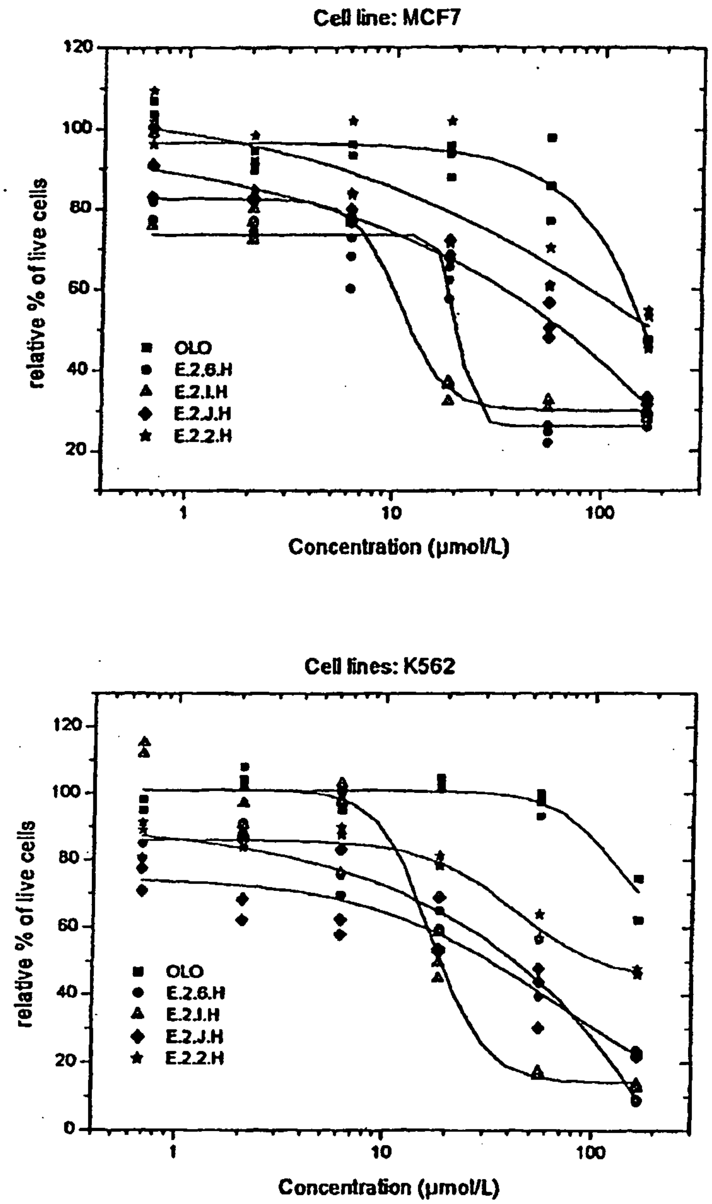

Cell line: MCF7
relative % of live cells
120
100
80
60
40
20
1
10
100
Concentration (µmol/L)
OLO
E.2.6.H
E.2.I.H
E.2.J.H
E.2.2.H
Cell lines: K562
relative % of live cells
120
100
80
60
40
20
0
1
10
100
Concentration (µmol/L)
OLO
E.2.6.H
E.2.I.H
E.2.J.H
E.2.2.H

Fig. 6

Description

[0001] This invention relates to new pyrazolo[4,3-d]pyrimidine derivatives and to their use in suitable utilities, especially in cancer therapy and agricultural practice.

[0002] The cell division cycle is an evolutionarily conserved process in all eukaryotic cells to control growth and division. The cell cycle consists of four distinct stages illustrated in Figure 7, the G1, S, G2 and M phase. Normal cellular proliferation is initiated and tightly controlled by a series of regulatory mechanisms that either permit or prevent cell cycle progression. In every phase, there are protein complexes, the cyclins and cyclin-dependent kinases regulating and advancing the cell cycle. Figure 7 shows the cell division cycle (cdc) consisting of four phases G1, S, G2 and M. Mitosis (the actual division) occurs in M-phase. In every phase, there are specific cyclin-dependent kinase complexes present (CDK's).

[0003] Proliferative disorders such as cancer are recognised as diseases of the cell cycle. It has been found that in tumour cells, the mechanisms that normally function to restrain cell division are defective, whilst those that promote division become more active. The genes responsible for these changes in growth and proliferation are generally named "tumour suppressors" and "oncogenes". Cell-cycle regulatory compounds are pivotal in the modulation of abnormal cellular proliferation as they provide ideal targets for therapy for a range of proliferative disorders.

A series of 3-,7-disubstituted pyrazolo[4,3-d]pyrimidines are useful for inhibition of cyclin-dependent kinases (preferably $p34^{cdc2}$/cyclin B). Hence they can be used as antimitotic and apoptotic drugs, particularly as anticancer drugs and herbicides. Likewise, the compounds can be used as anti-fungal agents, which may have high value in the treatment of aspergillosis, penicilliosis, actinomycosis and the like. Difference in homology of insect CDK genes permit selection of compounds of this invention which discriminate between insect/mammalian CDK enzymes and thus leads to insecticides.

Summary of the Invention

[0004] It is an object of this invention to provide antimitotic, anticancer, herbicidal, fungicidal and insecticidal compounds having improved selectivity and efficiency index, i.e. that are less toxic yet more efficacious than analogues known heretofore.

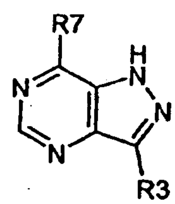
[0005] It is an object of this invention to provide 3,7-disubstituted pyrazolo[4,3-d]pyrimidines, which inhibit the cdks, cell proliferaton or block cytokinin receptors.

[0006] A further object of this invention is to provide a pharmaceutical composition, which comprises a 3,7-disubstituted pyrazolo[4,3-d]pyrimidine, and a pharmaceutically acceptable carrier.

[0007] A further object of this invention to provide a method for inhibiting cell proliferation and/or inducing apoptosis to a mammal or plant in need of an effective amount of 3,7-disubstituted pyrazolo[4,3-d]pyrimidines.

[0008] This invention further constitutes a method for inhibiting cell proliferation to a plant in need of an effective amount 3,7-disubstituted pyrazolo[4,3-d]pyrimidines.

[0009] The solution of this object are 3-, 7-disubstituted pyrazolo[4,3-d]pyrimidines represented by the general formula I



I

and pharmaceutically acceptable salts thereof, wherein

R3 is selected from the group consisting of

alkyl, cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl alkyl, cycloheteroalkyl, aryl, heterocycle, heteroaryl, arylalkyl, heteroarylalkyl, and heteroalkyl, wherein each of the groups may optionally be substituted,

R7 is selected from the group consisting of halogen, hydroxyl, hydroxylamino, amino, carboxyl, cyano, nitro, amido, sulfo, sulfamido, carbamino, unsubstituted or substituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted arylalkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroarylakyl, substituted

or unsubstituted cycloalkyl alkyl, substituted or unsubstituted cycloheteroalkyl alkyl;
R7'-X- wherein X is an -NH-, -N(alkyl)-, -O- or -S- moiety and
R7' is selected from the group consisting of H, alkyl, cycloalkyl, aryl, alkylcycloalkyl, arylalkyl, heterocycle, heterocycloalkyl, substituted alkyl, substituted cycloalkyl, substituted aryl, substituted arylalkyl, substituted heterocycle, substituted heteroaryl, substituted heteroarylalkyl, substituted heteroalkyl, substituted cycloalkyl alkyl and substituted cycloheteroalkyl alkyl.
If the above groups are substituted, they are preferably substituted by halogen, hydroxyl, amino, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, carbamino, alkyl, alkoxy, and/or substituted alkyl group, in particular by more than one of the above substituents, preferably by 1 to 3 substituents.

[0010] In another embodiment, this invention is a method for inhibiting cdks and cell proliferation and/or for inducing apoptosis in plants, comprising administering an effective amount of a composition comprising one or more compounds according to claim 1 to the plant. The cdk inhibiting molecules are useful for treating disorders, some of them involving cell proliferation, and thus are useful as herbicides.

[0011] In yet another embodiment, this invention is a pharmaceutical composition comprising one or more compounds according to claim 1 in an admixture with one or more pharmaceutical excipients.

[0012] In still another embodiment, this invention is a composition comprising one or more compounds according to claim 1 useful for treating fungal infections (fungi) in plants.

[0013] In another embodiment, this invention is a composition comprising one or more compounds according to claim 1 useful for treating insects and yeasts on plants.

[0014] 3,7-disubstituted pyrazolo[4,3-d]pyrimidines result in the acquisition of extremely high potency against plant viruses on the part of the defined compounds. As used herein, and unless modified by the immediate context:

"Halogen" preferably refers to fluorine, bromine, chlorine and iodine atoms.

"Hydroxy" refers to the group -OH.

"Mercapto" refers to group -SH.

"Alkyl" preferably refers to branched or unbranched C_1-C_8 alkyl chain which is saturated or unsaturated. Thus, the term "alkyl" when used herein encompasses alkyl alkenyl and alkinyl groups. Alkenyl groups preferably have 2 to 8 carbon atoms, alkinyl groups preferably have 3 to 8 carbon atoms. Such groups as methyl, ethyl, propyl, isopropyl, n-butyl, isobutyl, tert-butyl, allyl, ethinyl, propargyl, and the like can exemplify this term.

"Substituted alkyl" preferably refers to alkyl as described above including one to six, in particular 1 to 3 substituents such as hydroxyl, mercapto, alkylthio, halogen, alkoxy, acyloxy, amino, acylamino, hydrazino, carbamoyl, amido, carboxyl, sulfo, acyl, guanidino and the like. These groups may be attached to any carbon atom of the alkyl moiety.

"Alkoxy" denotes the group -OR, where R is preferably alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl or substituted cycloheteroalkyl as defined herein.

"Alkylthio" denotes the group -SR, where R is preferably as defined for "alkoxy" group.

"Sulfo" denotes the group —SO_3R, where R is preferably H, alkyl or substituted alkyl as defined above.

"Sulfamido" denotes to the group SO_2NRR' where R and R' is preferably H, alkyl or substituted alkyl as defined above.

"Acyl" denotes groups -C(O)R, where R preferably is alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl as defined herein.

"Aryloxy" denotes groups -OAr, where Ar is preferably an aryl, substituted aryl, heteroaryl or substituted heteroaryl group as defined herein.

"Alkylamino" denotes the group -NRR', where R and R'may independently be hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl or substituted heteroaryl as defined herein.

"Amido" denotes the group -C(O)NRR', where R and R'may independently be hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl as defined herein.

"Carboxyl" denotes the group -C(O)OR, where R is preferably hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, hetaryl or substituted hetaryl as defined herein.

"Acylamino denotes the group —NHCOR, where R may be alkyl, substituted alkyl, heterocycle, aryl, substituted aryl, heteroaryl and substituted heteroaryl as defined herein.

Carbamoylamino denotes the group NHCOOR, where R is preferably alkyl or aryl.

"Aryl" or "Ar" refers to an aromatic carbocyclic group having at least one aromatic ring (e.g., phenyl or biphenyl) or multiple condensed rings in which at least one ring is aromatic (e.g., 1,2,3,4-tetrahydronaphthyl, naphthyl, anthryl, or phenanthryl). Preferably, the aryl group has more than six, in particular 6 to 10 carbon atoms.

"Substituted aryl" refers to aryl as described above which is optionally substituted with one or more functional groups, in particular 1 to 3 substituents, such as halogen, alkyl, hydroxy, amino, acylamino, carbamoylamino, hydrazino, mercapto, alkoxy, alykthio, alkylamino, amido, carboxyl, nitro, sulfo and the like as defined herein.

"Heterocycle" refers to a unsaturated or aromatic carbocyclic group preferably having 1 to 3 rings and having at least one, preferably 1 to 3 and in particular 1 or 2 hetero atoms, such as N, O or S, within the ring; the ring can be single (e.g. pyranyl, pyridyl or furyl) or multiple condensed (e.g., quinazolinyl, purinyl, quinolinyl or benzofuranyl) which can optionally be unsubstituted or substituted with, e.g., halogen, amino, hydroxy, cyano, nitro, mercapto, alkoxy, alkylamino, acylamino, carbamoylamino, acyloxy, dialkylamino, alkylthio carboxyl, amido, sulfo, sulfamido, and the like as defined above. The heterocycle groups preferably has 5 to 10 ring atoms, which are either cabon atoms or hetero atoms as defined above.

"Heteroaryl" refers to a heterocycle in which at least one heterocyclic ring is aromatic.

"Substituted heteroaryl" refers to a heterocycle optionally mono or poly substituted with one or more functional groups, preferably 1 to 6, in particular 1 to 3 substituents, e.g., halogen, amino, hydroxy, cyano, nitro, mercapto, alkoxy, alkylamino, acylamino, carbamoylamino, acyloxy, dialkylamino, alkylthio carboxyl, amido, sulfo, sulfamido, and the like.

"Arylalkyl" refers to the group -R-Ar where Ar is an aryl group and R is alkyl or substituted alkyl group as defined above. The aryl groups can optionally be unsubstituted or substituted as defined above with, e.g., halogen, amino, acylamino, carbamoylamino, hydrazino, acyloxy, alkyl, hydroxyl, alkoxy, alkylthio, alkylamino, amido, carboxyl, hydroxy, aryl, nitro, mercapto, sulfo and the like.

"Heteroalkyl" refers to the group -R-Het where Het is a heterocycle group and R is a alkyl group as defined above. Heteroalkyl groups can optionally be unsubstituted or substituted as defined above with e.g., halogen, amino, hydroxy, cyano, nitro, mercapto, alkoxy, alkylamino, acylamino, carbamoylamino, acyloxy, dialkylamino, alkylthio, carboxyl, amido, sulfo, sulfamido, and the like.

"Heteroarylalkyl" refers to the group -R-HetAr where HetAr is an heteroaryl group and R is alkyl or substituted alkyl as defined above. Heteroarylalkyl groups can optionally be unsubstituted or substituted as defined above with, e. g., halogen, alkyl, substituted alkyl, alkoxy, alkylthio, nitro, mercapto, sulfo and the like.

"Cycloalkyl" refers to a divalent cyclic or polycyclic alkyl group containing preferably 3 to 15 carbon atoms.

"Substituted cycloalkyl" refers to a cycloalkyl group comprising one or more substituents as defined above with, e.g., halogen, amino, hydroxy, cyano, nitro, mercapto, alkoxy, alkylamino, acylamino, carbamoylamino, acyloxy, dialkylamino, alkylthio, carboxyl, amido, sulfo, sulfamido, and the like.

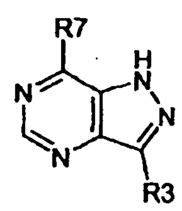
"Cycloheteroalkyl" refers to a cycloalkyl group as defined above wherein one or more, preferably 1 to 3, of the ring methylene group is replaced with a heteroatom (e.g., NH, O, S)

"Substituted cycloheteroalkyl" refers to a cycloheteroalkyl group as herein defined which contains one or more substituents as defined above, such as halogen, amino, hydroxy, cyano, nitro, mercapto, alkoxy, alkylamino, acylamino, carbamoylamino, acyloxy, dialkylamino, alkylthio, carboxyl, amido, sulfo, sulfamido and the like.

"Cycloalkyl alkyl" denotes the group -R-cycloalkyl where cycloalkyl is a cycloalkyl group as defined above and R is an alkyl or substituted alkyl as defined above. Cycloalkyl groups can optionally be unsubstituted or substituted as defined above with e.g., halogen, amino, hydroxy, cyano, nitro, mercapto, alkoxy, alkylamino, acylamino, carbamoylamino, acyloxy, dialkylamino, alkylthio, carboxyl, amido, sulfo, sulfamido and the like.

"Cycloheteroalkyl alkyl" denotes he group -R-cycloheteroalkyl where R is a alkyl or substituted alkyl as defined above and cycloheteroalkyl as as defined above. Cycloheteroalkyl groups can optionally be unsubstituted or substituted as defined above with e.g. halogen, amino, hydroxy, cyano, nitro, mercapto, alkoxy, alkylamino, acylamino, carbamoylamino, acyloxy, dialkylamino, alkylthio, carboxyl, amido, sulfo, sulfamido, and the like.

[0015] In a preferred embodiment the invention relates to 3-, 7-disubstituted pyrazolo[4,3-d]pyrimidines, which inhibit the cyclin-dependent and MAP kinases have formula I



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and the pharmaceutically acceptable acid salts thereof, wherein

R3 is

selected from an alkyl, cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, cycloheteroalkylalkyl, cycloalkyl alkyl, aryl, arylakyl, heteroaryl, heteroarylalkyl, wherein each of the groups may be optionally be subsituted by a halogen;

R7 is selected from the group consisting of halogen, hydroxyl, hydroxylamino, amino, hydrazino, carboxyl, cyano, nitro, amido, sulfo, sulfamido, carbamino, NHCONH2, NHC(=NH)NH2, alkyl, cycloalkyl, cycloalkylalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, cycloheteroalky, cycloheteroalkylalkyl, which is substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, acylamino, acyloxy, alkylamino, dialkylamino, alkylthio and carbamoyl group;

- R7'-X, wherein X is -NH-, -O-, -S- ;
- R7'-X, wherein X is preferably -N(alkyl)- selected at each occurrence from the group methyl, ethyl, propyl, isopropyl, ethinyl, allyl, propargyl, and isopent-2-en-1-yl;

R7' is

- C_1-C_8 branched or unbranched alkyl, alkenyl or alkinyl preferentially selected from the group methyl, ethyl, isopropyl, butyl, isobutyl, allyl, propargyl, isopent-2-en-1-yl, which is substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, acylamino, acyloxy, alkylamino, dialkylamino, alkylthio and carbamoyl group;
- acyl, -C(O)R, wherein R_a is C_1-C_6 branched or unbranched alkyl, alkenyl or alkinyl preferentially selected from the group methyl, ethyl, isopropyl, butyl, isobutyl, allyl, propargyl, isopent-2-en-1-yl, and 2-methylallyl, which is substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, acylamino, acyloxy, alkylamino, dialkylamino, alkylthio and carbamoyl group;
- amido, -C(O)NR_bR_c, wherein R_b and R_c is independently H, C_1-C_6 branched or unbranched alkyl, alkenyl or alkinyl preferentially selected from the group methyl, ethyl, isopropyl, butyl, isobutyl, allyl, propargyl, which is substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, acylamino, acyloxy, alkylamino, dialkylamino, alkylthio and carbamoyl group;
- sulfo, -SO_3R_d, wherein R_d is H, C_1-C_6 branched or unbranched alkyl, alkenyl or alkinyl preferentially selected from the group methyl, ethyl, isopropyl, butyl, isobutyl, allyl, propargyl, isopent-2-en-1-yl, and 2-methylallyl which is substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, acylamino, acyloxy, alkylamino, dialkylamino, alykthio and carbamoyl group;
- cykloalkyl is C_3-C_{15} cycloalkyl is preferentially selected from the group cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl or adamantyl;
- substituted cycloalkyl is C_3-C_{15} cycloalkyl is preferentially selected from the group cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl or adamantyl substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, acylamino, acyloxy, alkylamino, dialkylamino, alkylthio and carbamoyl group;
- cycloalkyl alkyl is R_f(cycloalkyl), wherein R_f is
    - C_1-C_6 alkyl, alkenyl or alkinyl preferentially selected from the group methyl, ethyl, isopropyl, butyl, allyl, propargyl, isopent-2-en-1-yl and 2-methylallyl, which is substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, acylamino, acyloxy, alkylamino, dialkylamino, alkylthio and carbamoyl group,
    - cykloalkyl is C_3-C_{15} cycloalkyl is preferentially selected from the group cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl or adamantyl;
    - substituted cycloalkyl is C_3-C_{15} cycloalkyl is preferentially selected from the group cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl or adamantyl substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, acylamino, acyloxy, alkylamino, dialkylamino, alkylthio and carbamoyl group;
    - aryl is preferentially selected from the group phenyl, biphenyl, naphthyl, tetrahydronaphtyl, fluorenyl, indenyl or fenanthrenyl substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, acylamino, acyloxy, alkylamino, dialkylamino, alkylthio and carbamoyl group;
    - heterocycle is preferentially selected from the group thienyl, furyl, pyranyl, pyrrolyl, imidazolyl, pyrazolyl, py-

ridyl, pyrazinyl, pyrimidinyl, pyridazinyl, isothiazolyl, isoxazyl substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, acylamino, acyloxy, alkylamino, dialkylamino, alkylthio and carbamoyl group;

- heteroalkyl is $-R_g$-Het, wherein
  - R_g is C_1-C_6 alkyl, alkenyl or alkinyl preferentially selected from the group methylen, 1,2-ethyliden, 1,3-propiliden, 1,4-butyliden, pentamethylen, hexamethylen, ethylendiyl, allyl-1,3-diyl, methylethan-1,1-diyl, methylethan-1,2-diyl, butan-1,3-diyl, which is substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, cyano and
  - Het is preferentially selected from the group thienyl, furyl, pyranyl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, isothiazolyl, isoxazyl substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, acylamino, acyloxy, alkylamino, dialkylamino, alkylthio and carbamoyl group;
- heteroaryl is $-R_h$-HetAr, wherein
  - R_h is C_1-C_6 alkyl, alkenyl or alkinyl preferentially selected from the group methylen, 1,2-ethyliden, 1,3-propiliden, 1,4-butyliden, pentamethylen, hexamethylen, ethylendiyl, allyl-1,3-diyl, methylethan-1,1-diyl, methylethan-1,2-diyl, butan-1,3-diyl, which is substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, cyano;
  - HetAr is preferentially selected from the group benzothienyl, naphthothienyl, benzofuranyl, chromenyl, indolyl, isoindolyl, indazolyl, qinolyl, isoqinolyl, ftalazinyl, qinaxalinyl, cinnolinyl, qinazolinyl substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, acylamino, acyloxy, alkylamino, dialkylamino, alkylthio and carbamoyl group;
- arylalkyl is $-R_i$Ar, wherein
  - R_i is C_1-C_6 alkyl, alkenyl or alkinyl preferentially selected from the group group methylen, 1,2-ethyliden, 1,3-propiliden, 1,4-butyliden, pentamethylen, hexamethylen, ethylendiyl, allyl-1,3-diyl methylethan-1,1-diyl, methylethan-1,2-diyl, butan-1,3-diyl, which is substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, cyano;
  - Ar is preferentially selected from the group phenyl, biphenyl, naphthyl, tetrahydronaphtyl, fluorenyl, indenyl or fenanthrenyl substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, acylamino, acyloxy, alkylamino, dialkylamino, alkylthio and carbamoyl group;
- cycloheteroalkyl is preferentially selected from the group piperidinyl, piperazinyl, morfolinyl, pyrrolidinyl, imidazolidinyl substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, acylamino, acyloxy, alkylamino, dialkylamino, alkylthio and carbamoyl group;
- cycloheteroalkyl alkyl, $-R_j$(cycloheteroalkyl), wherein
  - R_j is arylalkyl $-R_i$Ar, wherein
    - R_i is C_1-C_6 alkyl, alkenyl or alkinyl preferentially selected from the group group methylen, 1,2-ethyliden, 1,3-propiliden, 1,4-butyliden, pentamethylen, hexamethylen, ethylendiyl, allyl-1,3-diyl, methylethan-1,1-diyl, methylethan-1,2-diyl, butan-1,3-diyl, which is substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, cyano, and
    - Ar is preferentially selected from the group phenyl, biphenyl, naphthyl, tetrahydronaphtyl, fluorenyl, indenyl or fenanthrenyl substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, acylamino, acyloxy, alkylamino, dialkylamino, alkylthio and carbamoyl group, and
    - cycloheteroalkyl is preferentially selected from the group piperidinyl, piperazinyl, morfolinyl, pyrrolidinyl, imidazolidinyl substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido,

acylamino, acyloxy, alkylamino, dialkylamino, alkylthio and carbamoyl group;

- heteroarylalkyl is -R_k-HetAr, wherein
  - R_k is C_1-C_6 alkyl, alkenyl or alkinyl preferentially selected from the group group methylen, 1,2-ethyliden, 1,3-propiliden, 1,4-butyliden, pentamethylen, hexamethylen, ethylendiyl, allyl-1,3-diyl methylethan-1,1-diyl, methylethan-1,2-diyl, butan-1,3-diyl, which is substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, cyano, and

HetAr is preferentially selected from the group benzothienyl, benzofuranyl, chromenyl, indolyl, isoindolyl, indazolyl, qinolinyl, phthalazinyl, qinoxalinyl, qinazolinyl, karbazolyl, akridinyl, indolinyl, and isoindolinyl, which is substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, acylamino, acyloxy, alkylamino, dialkylamino, alkylthio and carbamoyl group.

[0016] The following derivatives are particularly preferred, namely: 7-(2-hydroxy-3-chlorobenzyl)amino-3-(methyl, ethyl, isopropyl, cyclopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-hydroxy-4-chlorobenzyl)amino-3-(methyl,ethyl,isopropyl, cyclopropyl, 3-pentyl benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-hydroxy-5-chlorobenzyl)amino-3-(methyl, ethyl, isopropyl, cyclopropyl, 3-pentyl.. benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-hydroxy-6-chlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-hydroxy-3-iodobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-hydroxy-4-iodobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-hydroxy-5-iodobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-hydroxy-6-iodobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-hydroxy-3-bromobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d] pyrimidine, 3-pentyl, 7-(2-hydroxy-4-bromobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d] pyrimidine, 7-(2-hydroxy-5-bromobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-hydroxy-6-bromobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-hydroxy-3-fluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-hydroxy-4-fluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-hydroxy-5-fluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 6-(2-hydroxy-6-fluorobenzyl) amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3-dihydroxy-4-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,5-dihydroxy-4-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,6-dihydroxy-3-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3-dihydroxy-3-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,5-dihydroxy-3-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,6-dihydroxy-4-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3-dihydroxy-4-chlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3-dihydroxy-4-chlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,5-dihydroxy-4-chlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,6-dihydroxy-4-chlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,6-dihydroxy-4-bromoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,6-dihydroxy-4-iodobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,6-dihydroxy-3-chlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl) pyrazolo[4,3-d]pyrimidine, 7-(2,6-dihydroxy-3-bromobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,6-dihydroxy-3-iodobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo [4,3-d]pyrimidine, 7-(2,6-dihydroxy-3-fluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d] pyrimidine, 7-(2,6-dihydroxy-3,5-dichlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,6-dihydroxy-3,5-dibromobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,6-dihydroxy-3,5-diiodobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,6-dihydroxy-3,5-difluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-fluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-fluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-fluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-bromobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-bromobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-bromobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-iodobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-iodobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-iodobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-chlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)

pyrazolo[4,3-d]pyrimidine, 7-(2-chlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-chlorobenzylamino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine 7-(4-chlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-acetylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-acetylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-acetylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-karboxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-karboxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-acetoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-acetoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-acetoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-nitrobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-nitrobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-nitrobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-cyanobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-cyanobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-cyanobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(5-nitro-2-methylbenzyl)amino-3-(methyl, ethyl, isopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-methylbenzyl)amino-3-(methyl, ethyl, isopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-methylbenzyl)amino-3-(methyl, ethyl, isopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-methylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-methylaminobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo [4,3-d]pyrimidine, 7-(3-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-hydroxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-hydroxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-hydroxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-hexylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-hexyloxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-ethoxybenzyl)amino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-ethoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-ethoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-ethylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-penthylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl) pyrazolo[4,3-d]pyrimidine, 7-(4-penthyloxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d] pyrimidine, 7-(4-fenoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-fenylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-propylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-propyloxybenzyl)aminopurin, 7-(4-oktylbenzylamino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-octyloxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine. 7-(4-ethyloxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3,4-diacetoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl) pyrazolo[4,3-d]pyrimidine, 7-(3,5-diacetoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d] pyrimidine, 67-(2,5-diaminobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3,5-dibromobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3,5-dibromo-4-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3-dichlorobenzyl) amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4-dichlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,5-dichlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,6-dichlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl) pyrazolo[4,3-d]pyrimidine, 7-(3,4-dichlorobenzyl)amino 3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3,5-dichlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3,4,5-tetrafluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-chloro-3,6-difluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(5-chloro-2-fluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3,4-trifluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3,5-trifluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4,5-trifluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3,4,5-trifluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl) pyrazolo[4,3-d]pyrimidine, 7-(2,3,6-trifluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d] pyrimidine, 7-(3-chloro-2,6-difluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-chloro-6-fluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,6-difluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4-difluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3,4-difluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,5-difluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3,5-difluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, ben-

zyl)pyrazolo[4,3-d]pyrimidine, 7-[5-fluoro-2-(trifluoromethyl)benzyl]amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl) pyrazolo[4,3-d]pyrimidine, 7-[4-fluoro-2-(trifluoromethyl)benzyl]amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl) pyrazolo[4,3-d]pyrimidine, 7-(2-chloro-5-(trifluoromethyl)benzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl) pyrazolo[4,3-d]pyrimidine, 7-(2-(difluoromethoxy)benzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo [4,3-d]pyrimidine, 7-(3-(difluoromethoxy)benzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-(difluoromethoxy)benzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[2-fluoro-5-(trifluoromethyl)benzyl]amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[3-fluoro-4-(trifluoromethyl)benzyl]amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[2-fluoro-4-(trifluoromethyl)benzyl]amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-(trifluoromethylthio)benzylamino)purin, 7-[2-fluoro-3-(trifluoromethyl)benzyl]amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-chloro-6-fluoro-3-methylbenzylamino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(6-chloro-2-fluoro-3-methylbenzylamino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[3-chloro-2-fluoro-5-(trifluoromethyl)benzyl]amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[3-chloro-2-fluoro-6-(trifluoromethyl)benzyl]amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3-difluoro-4-methylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,6-difluoro-3-methylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-fluoro-6-(trifluoromethyl)benzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-chloro-2,6-difluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[3-(trifluoromethylthio)benzyl]amino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl) pyrazolo[4,3-d]pyrimidine, 7-(3-fluoro-4-methylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo [4,3-d]pyrimidine, 7-[4-fluoro-3-methylbenzyl]amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(5-fluoro-2-methylbenzylamino)-3-(methyl, ethyl, isopropyl, 3-pentyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-chloro-3,6-difluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[4-(trifluoromethylthio)benzyl] amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-fluoro-5-(trifluoromethyl)benzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-chloro-4-fluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-(trifluoromethoxy)benzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-(trifluoromethyl) benzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[2-(trifluoromethyl)benzyl]amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-(trifluoromethyl)benzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[4-chloro-3-(trifluoromethyl)benzyl]amino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-fluoro-3-(trifluoromethyl)benzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[3,5-di(trifluoromethyl)benzyl]amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[3-(trifluoromethoxy)benzyl]amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[4-(trifluoromethoxy)benzyl]amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-(trifluoromethyl)benzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[4-(diethylamino)benzyl]amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3,4-dihydroxybenzyl]amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3,5-dihydroxybenzyl]amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3,4-dihydroxybenzyl]amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3-ethylenedioxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4-dihydroxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,5-dihydroxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, isopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,6-dihydroxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3,4-dimethoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3,4-dimethoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo [4,3-d]pyrimidine, 7-(3,5-dimethoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3-dimethoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4-dimethoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,5-dimethoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,6-dimethoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-hydroxy-4-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-hydroxy-3-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-hydroxy-3-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-hydroxy-4-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-hydroxy-2-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-hydroxy-5-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-hydroxy-4-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-hydroxy-3-methoxybenzylamino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl) pyrazolo[4,3-d]pyrimidine, 7-(2-hydroxy-6-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo [4,3-d]pyrimidine, 7-(3-hydroxy-5-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]

pyrimidine, 7-(4,5-dimethoxy-2-nitrobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3,4-dimethylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3-dimethylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4-dimethylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,6-dimethylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,6-dimethyl-4-hydroxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3,5-dimethyl-4-hydroxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-fluoro-4-hydroxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-fluoro-4-methylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3,4-dinitrobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl) pyrazolo[4,3-d]pyrimidine, 7-(3,5-dinitrobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-methyl-5-nitrobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-methyl-4-nitrobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3,4-diiodo-4-hydroxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-chloro-3,4-dimethoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-chloro-3,5-dinitrobenzyl) amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-chloro-4-fluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-chloro-4-fluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-chloro-6-methylbenzyl)amino-3-(methyl, ethyl, isopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-chloro-2-methylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-chloro-4-methylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d] pyrimidine, 7-(5-chloro-2-methoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-chloro-4-fluorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-chloromethylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-chloro-5-nitrobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-chloro-6-nitrobenzyl) amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-chloro-3-nitrobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(5-chloro-2-nitrobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 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benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-amino-6-chlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-amino-4-chlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-amino-3-chlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d] pyrimidine, 7-(2-amino-4-chlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-amino-6-chlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,6-diamino-3-chlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,6-diamino-4-chlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-amino-3-chlorobenzyl)amino-3-(methyl, ethyl, isopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-amino-5-dichlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(5-amino-2-methylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-amino-3-nitrobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-amino-3-nitrobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-benzyloxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-acetylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-acetylbenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3,4-trimethoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4,5-trimethoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4,6-trimethoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3,4,5-trimethoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4,6-trimethoxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3,4-trihydroxybenzy)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4,6-trihydroxybenzy)lamino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3,4-trihydroxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3,4,5-trihydroxybenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4,6-trihydroxybenzyl) amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4,5-trichlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4,5-trichlorobenzyl)amino-3-(methyl, ethyl, iso-

propyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4,6-trichlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3,4-trichlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3,5-trichlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3,6-trichlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,5,6-trichlorobenzyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-anilino-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[2,4-bis(trifluoromethyl)anilino]-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[2,5-bis(trifluoromethyl)anilino]-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[2,4-bis(trifluoromethyl)anilino]-3-(methyl, ethyl, isopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 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benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-bromo-2-fluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-bromo-4-fluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-bromo-4-methylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-bromo-2-methylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 6-(4-bromo-3-methylanilino))-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[2-bromo-4-(trifluoromethoxy)anilino]-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[3-bromo-4-(trifluoromethoxy)anilino]-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[4-bromo-2-(trifluoromethoxy)anilino]-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-bromo-4,5,6-trifluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 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3-pentyl, benzyl)

pyrazolo[4,3-d]pyrimidine, 7-(2-chloro-5-fluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-chloro-6-fluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-chloro-2-fluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-chloro-4-fluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-chloro-2-fluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(5-chloro-2-fluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-chloro-4-fluoro-5-methylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl) pyrazolo[4,3-d]pyrimidine, 7-(5-chloro-4-fluoro-2-nitroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo [4,3-d]pyrimidine, 7-(5-chloro-2-hydroxyanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-chloro-2-iodoanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl,benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-chloro-4-iodoanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-chloro-6-methylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-chloro-2-methylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[3-chloro-4-(trifluoromethoxy)anilino]-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[4-chloro-2-(trifluoromethoxy)anilino]-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-fluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-fluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-fluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo [4,3-d]pyrimidine, 7-(2-fluoro-4-iodoanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-fluoro-5-nitroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-fluoro-4-methylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-fluoro-2-methylanilino)-3-(methyl, ethyl, isopropyl, 3-cyklopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-fluoro-4-methylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-fluoro-2-methylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-fluoro-4-methylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(5-fluoro-2-methylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-fluoro-2-nitroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-fluoro-3-nitroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-jodoanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[2-fluoro-4-(trifluoromethyl)anilino]-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-iodo-2-methylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-methoxyanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-methoxyanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-methoxyanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-methoxy-5-methylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-methoxy-6-methylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-methoxy-2-methylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(5-methoxy-2-methylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 6-[4-methoxy-3-(trifluoromethyl)anilino]-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-methylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-methylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-methylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl) pyrazolo[4,3-d]pyrimidine, 7-[2-methyl-3-(trifluoromethoxy)anilino]-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[2-methyl-4-(trifluoromethoxy)anilino]-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[2-(methylthio)anilino]-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[4-(methylthio)anilino]-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl) pyrazolo[4,3-d]pyrimidine, 7-(2-nitroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d] pyrimidine, 7-(3-nitroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-nitroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-nitro-4,5,6-trifluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[2-nitro-4-(trifluoromethoxy)anilino]-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-nitrotetrafluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3,4,5,6-pentabromoanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 6-(2,3,4,5,6-pentafluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3,4,5-tetrachloroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3,5,6-tetrachloroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-(1,1,2,2-tetrafluoroethoxy)anilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3,4,5,-tetrafluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3,4,6,-tetrafluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3,5,6,-tetrafluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)anilino]-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo

[4,3-d]pyrimidine, 7-(2,4,6-tribromoanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4,6-tribromo-3,5-dijodoanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d] pyrimidine, 7-(2,3,4-trichloroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4,5-trichloroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4,6-trichloroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4,5-trifluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3,5-trifluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3,6-trifluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3,4-trifluoroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2-trifluoromethoxyanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-trifluoromethoxyanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-trifluoromethoxyanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,3,4-trifluoro-6-nitroanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4,5-trimethylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(2,4,6-trimethylanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-chloro-4-carboxyanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-carboxy-4-hydroxyanilino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-cyclohexylamino-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-cyclopentylamino-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-cyclobutylamino- 3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-allylamino-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-diallylamino-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine 7-isopentylamino-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3,3-dimethylallylamino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(3-hydroxymethyl-3-methylallyl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-propargylamino-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-furfurylamino-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(oxazol-4-yl)amino-3-(methyl, ethyl, isopropyl, 3-pentyl, cyclopropyl, benzyl) pyrazolo[4,3-d]pyrimidine, 7-(2-pyridylamino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d] pyrimidine, 7-(3-pyridylamino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-pyridylamino)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(4-morfolinyl)-3-(methyl, ethyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(1-chinuklidinyl)-3-(methyl, isopropyl, 3-pentyl, benzyl) pyrazolo[4,3-d]pyrimidine, 7-(1-etyleniminyl)-3-(methyl, ethyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(1-propyleniminyl)-3-(methyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(1-pyrolidinyl)-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(1-piperidinyl)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl) pyrazolo[4,3-d]pyrimidine, 7-(1-piperazinyl)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d] pyrimidine, 7-pyrazol-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-imidazol-3-(methyl, ethyl, isopropyl, 3-pentyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(1-imidazolinyl)-3-(methyl, ethyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine, 7-(1-pyrazolinyl)-3-(methyl, ethyl, isopropyl, 3-pentyl, cyklopropyl, benzyl)pyrazolo[4,3-d]pyrimidine.

[0017] The novel compounds of this invention per se or as intermediates in the preparation of novel compound having a wide variety of industrial utilities.

[0018] The compounds of the formula I and their pharmaceutically acceptable salts inhibit selectively the enzyme $p34^{cdc2}$/cyclin B kinase and related cdks (cdk1, cdk2, cdk5, cdk7, MAP kinases).

[0019] In another embodiment, this invention is a method for inhibiting cdks and cell proliferation and/or for inducing apoptosis in plants comprising administering an effective amount of the composition of claim 1 to the plant.

[0020] In still another embodiment, this invention is a composition useful for treating fungal infections (fungi) in humans, animals and plants.

[0021] Diubstituted pyrazolo[4,3-d]pyrimidine derivatives result in the acquisition of extremely high potency against viruses on the part of the defined compounds. An important aspect of the present invention is a method for inhibiting proliferation of a DNA virus dependent upon events associated with cell proliferation for replication. The DNA virus includes any of the retrovirus family. The efective amount is that sufficient to inhibit cellular CDK activity to extent impending viral replication.

[0022] In addition to other CDK1-related kinases, this kinase controls certain steps of cell division cycles, in particular the transition from G_1 phase into the S phase and in particular the transition from the G_2 phase into the M-phase. Out the basis of this findings, it can be expected that the compounds of the formula I and their acceptable salts can be used as antimitotic compounds and for treatment of proliferative diseases.

[0023] In addition to therapeutic applications it will be apparent the subject compounds can be used as a cell culture additive for controlling proliferative and/or differentiation states of cells *in vitro*, for instance, by controlling the level of activation of a CDK. By preventing the activation of a G_0/G_1 CDK, the subject inhibitors can prevent mitotic progression

and hence provide a means for ensuring an adequately restrictive environment in order to maintain cells at various stages of differentiations, and can be employed, for instance, in cell cultures designed to test the specific activities of trophic factors. Other tissue culture systems which require maintenance of differentiation will be readily apparent to those skilled in the art

[0024] It is likely that inhibition by the compounds, of the invention of the catalytic activity of cyclin-dependent kinases in mediated by interaction of the compounds at the ATP-binding site of the enzyme. Such compounds are particularly desirable for reducing excessive cell growth, since they allow inhibition of the kinase activity regardless of the cause underlying the excessive kinase activity leading to excessive cell proliferation. Thus, the compounds of the invention are active in situations in which the excessive kinase activity results from the kinase being a mutated hyperactive, form of the kinase and situations in which the kinase is present at excessive levels. Such compounds can also block excessive kinase activity in situations in which the cyclin regulating the kinase is present at excessive levels or its binding to the kinase is enhanced. Furthermore, compounds which block kinase activity by interacting with the ATP binding site of the enzyme are also useful for inhibiting kinase activity in situations in which a natural inhibitor of cyclin-kinase complexes is mutated.

[0025] It will also be aparent that differential screening assays can be used to select for those compounds of the present invention with specificity for CDK enzymes. Thus, compounds, which act specifically on eukaryotic pathogens, e.g., are anti-fungal or anti-parasitic agents, can be selected from the subject of the inhibitors.

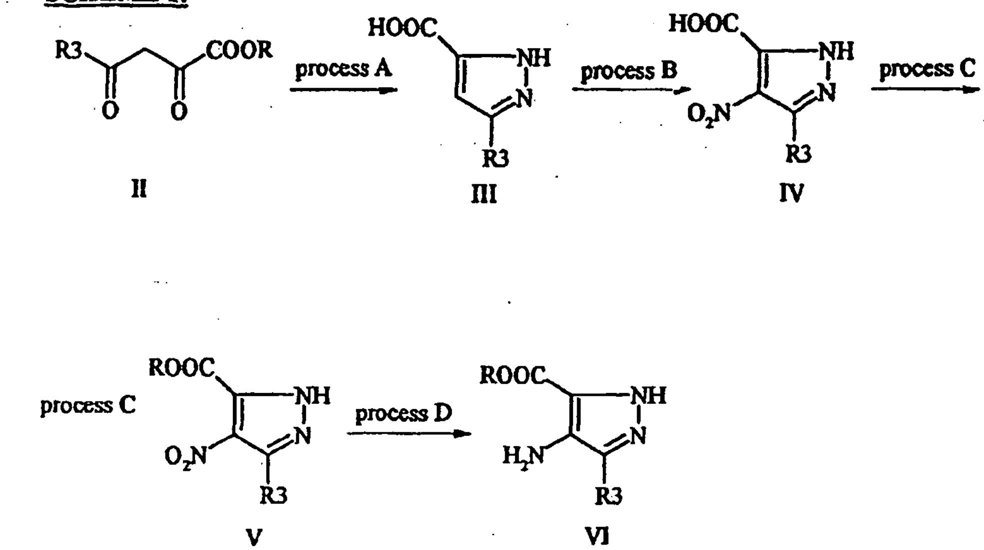
[0026] By way of illustration, the assays described in the art can be used to screen for agents which may ultimately be useful for inhibiting at leas one fungus implicated in such mycosis as aspergillosis, blastomycosis, chromoblastomycosis, coccidiomycosis, conidiosporosis, actinomycosis, penicillosis, monoliasis, or sporotrichosis. For example, if the mycotic infection to which treatment is desired is aspergillosis, an assay as described above or in the appended examples can comprise comparing the relative effectiveness of a test compound on inhibiting a plant CDK enzyme with its effectiveness towards a CDK enzyme from yeast. Likewise, the differential screening assays can be used to identify anti-fungal agents which may have value in the treatment of aspergillosis by making use of the CDK genes cloned from yeast such as *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Aspergillus nidulans*, or *Apergillus terreus*.

[0027] In yer another embodiment, certain of the subject CDK inhibitors can be selected on the basis of inhibitory specificity for plant CDK-s relative to the mammalian enzyme. For example, a plant CDK can be sidposed in a differential screen with one or more of the human enzymes to select those compounds of greatest selectivity for inhibiting the plant enzyme. Thus, the present invention specifically contemplates formulations of the subject CDK inhibitors for agricultural applications, such as in the form of a defoliant or the like.

PROCESSES FOR PREPARATION

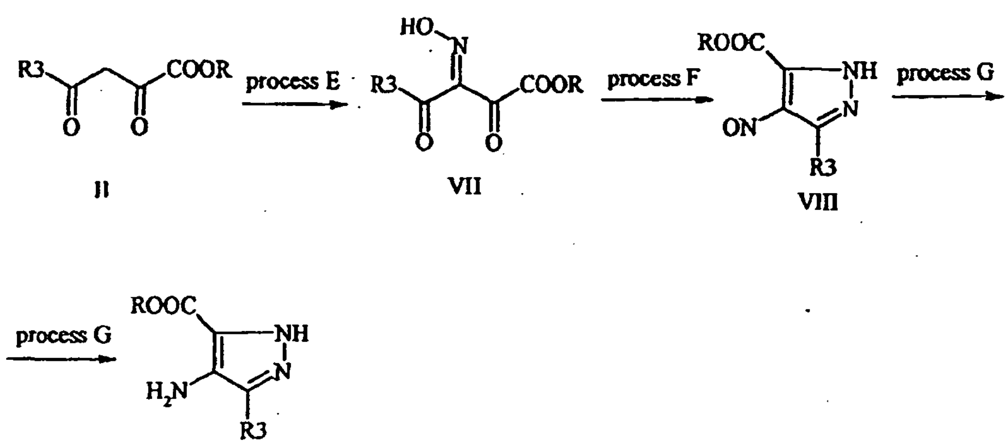
[0028]

SCHEME 1:



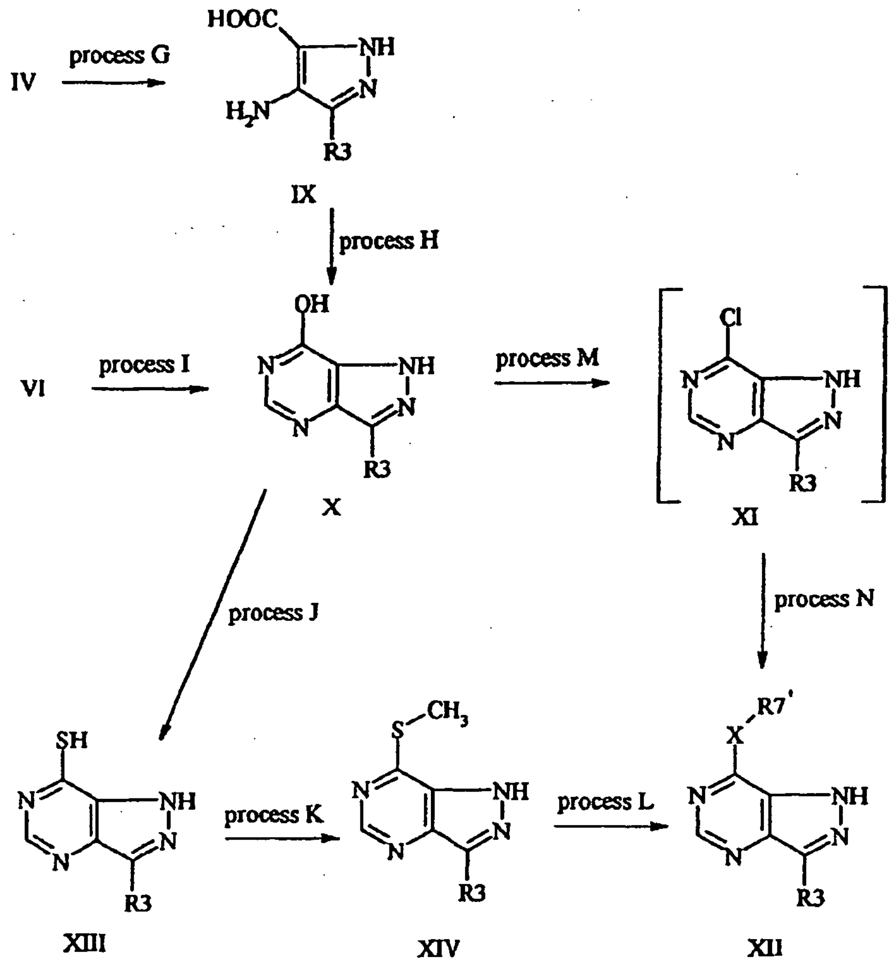
process A: $N_2H_4 \cdot H_2O$/T=96°C
process B: H_2SO_4/HNO_3
process C: ROH /HCl
process D: RaNi + H_2 / CH_3OH+H_2O; Pd or Pt + H_2/ CH_3OH+CH_3COOH, $SnCl_2$; $S_2O_4^{2-}$

SCHEME 2:



process E: $NaNO_2$ / HCl in C_2H_5OH or N_2O_3 (g)
process F: N_2H_4 H_2O
process G: $Na_2S_2O_4$ / $EtOAc+H_2O$

SCHEME 3:



process G: $Na_2S_2O_4$ / EtOAc+H_2O
process H: formamide/ reflux
process I: formamidin acetate/ Et_3N/ 2-ethoxyethanol/ 90°C
process J: P_2S_5/ pyridine/ reflux
process K: CH_3I/ 1 M KOH
process L: R7'NH_2 or R7'ONa (K,Li) or R7'SNa (K,Li))
process M: $SOCl_2$/$CHCl_3$/ DMF /T=80°C or $POCl_3$ or $POCl_3$ / PCl_5
process N: R7'NH_2 or R7'ONa (K,Li) or R7'SNa (K,Li))

[0029] In one approach the 3-isopropyl-7-substituted pyrazolo[4,3-d]pyrimidine of the formula I, wherein R7 substituents are defined above for a compound of the formula I, are prepared by reaction of 7-chloro-3-isopropylpyrazolo [4,3-d]pyrimidine with appropriate nucleophile as 1)amine (amonium hydroxyde, hydrazine, hydroxylamine, benzylamine; 2-, 3-, 4-hydroxybenzylamine; dihydroxybenzylamine; 3-chloroaniline, etc.) or 2) lithium(natrium, kalium) salt of alcohole or mercaptane. Preferably, the appropriate nucleophil reactant may be R7'-X-Y, wherein R7'-X- is as defined in claim 1 and Y is H. A nucleophil is reagent is able to attack a place in molecule with absence of electrons. An

appropriate alkylating agent is a reagent which is source of carbo cations which which attack a place in a molecule with excess of electrons - preferentially free electron couples, usually oxygen, nitrogen and sulfur, such as R7'-Z, wherein R7' is as defined in claim 1 and Z is selected from halogen, toluensulfonate, and mesylate. 7-Chloro-3-isopropylpyrazolo[4,3-d]pyrimidine is disolved in chloroform and appropriate $R^{7'}$-NH_2 or $R^{7'}$-O(Li, Na,K), or R7'-S(Li, Na,K) (5 - 20 eq.) was added. After heating for several hours, the reaction mixture is cooled and the 7-substituted-3-isopropylpyrazolo[4,3-d]pyrimidine is obtained.

[0030] In another approach the 3-ethyl substituted pyrazolo[4,3-d]pyrimidine of the formula I, wherein R7 substituents are defined above for a compound of the formula I, are prepared by reaction of 7-chloro-3-ethylpyrazolo[4,3-d]pyrimidine with appropriate nucleophile as 1)amine (amonium hydroxide, hydrazine, hydroxylamine, benzylamine; 2-, 3-, 4-hydroxybenzylamine; dihydroxybenzylamine; 3-chloroaniline, etc.) or 2) lithium(natrium, kalium) salt of alcohole or mercaptane. 7-Chloro-3-isopropylpyrazolo[4,3-d]pyrimidine is disolved in chloroform and appropriate $R^{7'}$-NH_2 or $R^{7'}$-O(Li, Na,K), or $R^{7'}$-S(Li, Na,K) (5 - 20 eq.) was added. After heating for several hours, the reaction mixture is cooled and the 7-substituted-3-ethylpyrazolo[4,3-d]pyrimidine is obtained.

[0031] In yet another approach the 3-methyl substituted pyrazolo[4,3-d]pyrimidine of the formula I, wherein R7 substituents are defined above for a compound of the formula I, are prepared by reaction of 7-chloro-3-methylpyrazolo [4,3-d]pyrimidine with appropriate nucleophile as 1)amine (amonium hydroxide, hydrazine, hydroxylamine, benzylamine; 2-, 3-, 4-hydroxybenzylamine; dihydroxybenzylamine; 3-chloroaniline, etc.) or 2) lithium(natrium, kalium) salt of alkohole or mercaptane. 7-Chloro-3-isopropylpyrazolo[4,3-d]pyrimidine is disolved in chloroform and appropriate $R^{7'}$-NH_2 or $R^{7'}$-O(Li, Na,K), or $R^{7'}$-S(Li, Na,K) (5 - 20 eq.) was added. After heating for several hours, the reaction mixture is cooled and the7-substituted-3-methylpyrazolo[4,3-d]pyrimidine is obtained.

[0032] Further, Fig. 1 shows a diagram displaying the specific inhibition of plant cdc2 kinase activity in plant cells. Enzyme activity bound to $p13^{suc1}$-agarose was measured by phosphorylation of histone H1 substrate protein in the presence of various concentrations of (1) 7-furfurylamino-3-methylpyrazolo[4,3-d]pyrimidine, (2) 7-benzylamino-3-isopropylpyrazolo[4,3-d]pyrimidine, (3) 7-(3-chloroanilino)-3-isopropylpyrazolo[4,3-d]pyrimidine, (4) 7-(3-hydroxybenzyl) amino-3-isopropylpyrazolo[4,3-d]pyrimidine.

[0033] Fig. 2 shows pictures of the induction of aberrant mitosis in root meristem cells of *V. faba* after the treatment with 200 mM A2.16.32, wherein

pictures a-e - control cells
pictures a'-e' - cells treated with 200 mM A2.16.32 for 12 hr.
picturre a - lower magnification shouwing frequency od mitosis (a), and aberrant mitosis (a'). b,b' - prophase, c,c' - metaphase, d,d' - anaphase, e,e' - telophase.

[0034] Fig. 3 shows immunofluorescence visualization of microtubules in control (A) and treated (B) root meristem cells of *V.faba*. Green - FITC immunolabelling for alfa-tubulin (left column), red - immunolabelling for gamma-tubulin (middle column), blue- immunolabelling for DNA labelling with DAPI (right column).

[0035] Fig. 4 shows pictures of the electrophoretic detection of double strand DNA breaks (marker of apoptosis) after the treatment of root meristem cells of *Vicia faba* with bohemine (200 μM) wherein the slots are

1. molecular weight markers
2. control I - DNA after 10 h incubation without the drug
3 DNA after 10 h incubation with bohemine
4. control II - DNA after 44 hod inkubation without the drug and
5. DNA pafter 44 hod incubation with bohemine

[0036] Fig 5 shows immunofluorescence detection of DNA double strand breaks in root meristem cells of *V.faba* treated with bohemine.

A. Root meristem cells of *V.faba*, treated with bohemine (200 mM). 1st line
B. Control cells. 2nd. line

1. column - FITC labelling of DNA breaks.
2 column - DAPI labelling for chromatin
3. column - merged images of FITC and DAPI

The following EXAMPLES serve to illustrate the invention without limiting the scope thereof.

[0037] The starting material for the compounds of the formula I is available from commercial sources (Sigma, Aldrich,

Fluka, etc.). Melting points were determined on a Koffler block and are uncorrected. Evaporations were carried out on a rotary evaporator under vacuum at temperatures below 80°C. The ^{1}H NMR spectra (σ, ppm; J, Hz) were measured on Varian VXR-400 (400MHz) or on Varian Unity 300 (400MHz) instruments. All spectra were obtained at 25°C using tetramethylsilane as on a internal standard. Electron impact mass spectra m/z (rel.%, composition, deviation) were measured on a VG 7070E spectrometer (70eV, 200°C, direct inlet) Quadrupole mass spectra were measured on a Micromass ZMD detector with electrospray ionization. Merck silica gel Kieselgel 60 (230-400 mesh) was used for column chromatography. All compounds gave satisfactory elemental analyses (±0,4%).

EXAMPLE 1:

methyl 2,4-dioxo-5-methylhexenoate (II): Prepared according to :

[0038]

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EXAMPLE 2:

5-isopropylpyrazol-3-carbozylic acid (III)

[0039] A solution of methyl 2,4-dioxo-5-methylhexenoate II (31g; 180 mmol) and $N_2H_4 \cdot H_2O$ (13mL, 180 mmol) in ethanol (120 mL) was refluxed for 2 hours. The mixture was poured into H_2O and extracted with chloroform. The chloroform extract was concentraded under reduced pressure to give a yellow liquid The liquid and aq. 3 M NaOH (120 mL) was stirred overnight at room temperature. The acidification of the yellow solution to pH=2 with conc. HCl affords crystals. Crystals were filtered off and washed with ice-water. Yield 68%, mp=136-140°C.
^{1}H NMR(300MHz, MeOD): 1.14d(6H, 7.1Hz), 3.02sept.(1H, 7.1Hz), 6.59s(1H); CHN required C=54.54%; H=6.54%; N=18.17%; found: C=54.58%; H=6.38%, N=18.12%

EXAMPLE 3:

5-isopropyl-4-nitropyrazole-3-carboxylic acid (IV):

[0040] To an ice-cooled and stirred solution of 2.9g (18.8mmol) 5-isopropylpyrazol-3-carboxylic acid III in fuming sulphuric acid 1 mL (65%,), sulphuric acid 7.6 mL (100%) and the nitric acid 3 mL (65%) was added portionwise. The stirring was continued for 1h at room temperature and then another 3h at 104°C temperature and then poured into ice-water. The white precipitate of product was filtered and crystallized from water; (yield 76%), mp=139-142°C; ^{1}H NMR (300MHz, DMSO): 1.22d(6H, 7.1Hz), 2.94sept(1H, 7.1Hz), 3.33s(1H), 6.45bs(1H); CHN required. C=42.02%; H=4.56%; N=21.09%; found: C=42.41%, H=4.49%; N=21 01%.

EXAMPLE 4:

Methyl 5-isopropyl-4-nitropyrazol-3-carboxylate (V)

[0041] 5-Isopropyl-4-nitropyrazole-3-carboxylic acid was added to a 4 5M solution of HCl in absolute methanol. The reaction mixture was heated at 60°C for 7 hours and then was evaporated to dryness The title compound was crystallised from ethyl acetate; yield 91%; mp=78-80°C. ^{1}H NMR (300MHz, $CDCl_3$): 1.39d(6H, J=7.1Hz); 3.64sept(1H, J=7 1Hz), 3.98s(3H). CHN required. C=45.07%, H=5.20%; N=19.70% ; found: C=45.21%; H=5.23%; N=19 65%.

EXAMPLE 5:

Methyl 4-amino-5-isopropylpyrazol-3-carboxylate (VI)

mode A

[0042] To a solution of methyl 5-isopropyl-4-nitropyrazol-3-carboxylate (4.34g, 24 mmol) in 20 mL ethanol and 5 mL water was added 1g RaNi (an activity W5). The mixture was stirred under hydrogen atmosphere (760 Torr) for 12 hours. The RaNi was filtered off and the filtrate was concenrated in vacuo. The residue crystals were washed with cooled ethylacetate; yield 95%; mp=122-123°C. MS(EI, 70eV, direct inlet): 183(88; $C_8H_{13}N_3O_2^{\cdot+}$; -1.0), 168(59), 152(3), 136

(100), 108(8), 80(16), 68(20). [1]H NMR(400MHz, $CDCl_3$): 1.31d(6H; J=6.9Hz), 2.93sept(1H; J=6.9Hz), 3.9s(3H). IR (KBr, cm^{-1}): 3399, 3296, 1710, 1626, 1584,1302.

mode B

[0043] To a solution of methyl 4-nitropyrazol-3-carboxylate V (7.34g, 34.4mmol) in 36 mL n-propanole, 6 mL water and 5.6 mL 10 M HCl was added 0.55g PtO_2. The mixture was stirred under hydrogen atmosphere (760 Torr) for 9 hours. The reaction mixture was filtered and the filtrate was concentrated to dryness in vacuo. The desired amine was liberated by treatment of aq. ammonia during extraction into chloroform. The product crystallised after evaporation; yield 95%; mp=122-123°C.

EXAMPLE 6:

7-hydroxy-3-isopropylpyrazolo[4,3-d]pyrimidine (X)

[0044] A mixture of aminoester VI (1.5 g, 8.42 mmol) , formamidine acetate (2.47 g, 24 mmol) and triethylamine (5,25 mL) in 32 mL of 2-ethoxyetanol was heated for 2 hours at 90°C under argon atmosphere. The excess of triethylamine was evaporated from cellosolve solution in vacuo, the crystallised product was filtered off and washed with chloroform. An analytical sample was obtained by recrystallisation from ethanol. Yield 96%; mp=302-304°C. [1]H-NMR (300MHz, CD_3OD): 1.41d(6H, J=7.15Hz), 3.40sept(1H, J=7.15Hz), 7.82s(1H). [13]C-NMR (400MHz, DMSO-d_6+AcOD): 21.912, 25.985, 141.85, 172.17. CHN required: C=53.92%; H=5.66%; N=31.44; found: C=53.20%; H=5.58%; N=31.39%. MS (EI): 178(35; $C_8H_{10}N_4O.^+$); 177(8) 163(25;$C_7H_7N_4O.^+$); 150(18;$C_6h_6N_4O.^+$); 54(18); 53(10); 41(11), 28(38); 27(11).

EXAMPLE 7:

[7-chloro-3-isopropylpyrazolo[4,3-d]pyrimidine (XI)]

[0045] 7-Hydroxy-3-isopropylpyrazolo[4,3-d]pyrimidine X (200 mg, 1.122 mmol) was dissolved in the mixture of 0.81 mL (11 mmol) of thionyl chloride, 0.12 mL (1.56 mmol) of dimethylformamide and 5 mL of chloroform. This mixture was heated under reflux for 3 hours. The solution was evaporated to dryness in vacuo and the residue was dissolved in chloroform. This solution was extracted twice with a small portions of water and combined chloroform extract was dried over Na_2SO_4. This compound VIII was not isolated and was used immediately as chloroform solution in following reaction step.

EXAMPLE 8:

7-benzylamino-3-isopropylpyrazolo[4,3-d]pyrimidine XIIa

[0046] To chloroform solution of XI(prepared in the subsequent step from 200 mg X) was added 3 mL of benzylamine. This mixture was stirred at room temperature 10 minutes and then evaporated to dryness in vacuo. The crude product was purified by columm chromatography on silica gel, the mixture of chloroform/methanol (98.5 / 1.5) was used as mobile phase. Yield 82%; mp=153-154°C. [1]H-NMR (400MHz, $CDCl_3$): 1.40d(6H, J=7.02Hz); 3.41sept(1H, J=7.02Hz); 4.80s(2H), 7.25m(5H), 6.57s (1H), 8.4s (1H). CHN required: C=67.39%; H=6.41%; N=26.20; found: C=67.33%; H=6.43%; N=26.24%. MS (EI): 267(62;$C_8H_{10}N_4O.^+$); 266(18) 252(44); 239(6); 106(49); 91(100); 65(21); 43(14); 41 (16).

EXAMPLE 9:

7-(2-hydrozybenzyl)amino-3-isopropylpyrazolo[4,3-d]pyrimidine XIIb

[0047] To chloroform solution of XI (prepared in the subsequent step from 200 mg X) was added 1 31 mL (7.7 mmol) *N*-ethyldiisopropylamine, 3 mL EtOH and 500 mg (4.06 mmol) 2-hydroxybenzylamine This mixture was heated one hour at 60°C and then was evaporated to dryness in vacuo The crude product was purified by chromatography on silica gel in the mixture of chloroform/methanol/AcOH (20.0.4.0.1). Yield 40%; mp=214-217°C. [1]H-NMR (400MHz, DMSO-d_6): 1.36d(6H, J=6.96Hz), 3.28sept(1H, J=6.96Hz); 4.65s(2H), 6.78-7 26m(4H), 8.21s (1H). CHN required: C=63.59%; H=6.05%, N=24.72; found : C=63 39%; H=6.07%; N=24.62%. MS (ES). $[M+H]^+$=274.3 (100).

EXAMPLE 10:

7-(3-hydroxybenzylamino)-3-isopropylpyrazolo[4,3-d]pyrimidine XIIc

[0048] To chloroform solution of XI (prepared in the subsequent step from 200 mg X) was added 1.5 mL (8.8 mmol) *N*-ethyldiisopropylamine, 5 mL EtOH and 500 mg (4.06 mmol) 3-hydroxybenzylamine. This mixture was heated one hour at 60°C and then was evaporated to dryness in vacuo. The crude product was purified by column chromatography on silica gel in the mixture of chloroform/methanol/AcOH (20:0.6:0.1). Yield 48%; mp=220-221°C. ^{1}H -NMR (300MHz, DMSO-d_6): 1.38d(6H, J=7.14Hz); 3.26sept(1H, J=7.14Hz); 4.68s(2H), 6.62-7.17m(3H), 8.22s (1H); 9.31s(1H). MS (ES): $[M+H]^+$=274 3 (100).

EXAMPLE 11:

7-(4-hydroxybenzyl)amino-3-isopropylpyrazolo[4,3-d]pyrimidine XIId

[0049] To chloroform solution of XI (prepared in the subsequent step from 200 mg X) was added 1 5 mL (8.8 mmol) *N*-ethyldiisopropylamine, 5 mL EtOH and 500 mg (4.06 mmol) 4-hydroxybenzylamine. This mixture was heated one hour at 60°C and then was evaporated to dryness in vacuo. The crude product was purified by column chromatography on silica gel in the mixture of chlorofom-/methanol/AcOH (20:1:0.1). Yield 49%; mp=234-236°C. ^{1}H-NMR (300MHz, DMSO-d_6): 1.28d(6H, J=6.59Hz); 3.58sept(1H, J=6.59Hz); 4 60s(2H), 6.73-7.21m(4H), 8.20s (1H) MS (ES): $[M+H]^+$=274.3 (100)

EXAMPLE 12:

7-(3-chloroanilino)-3-isopropylpyrazolo[4,3-d]pyrimidine XIIe

[0050] To chloroform solution of XI (prepared in the subsequent step from 200 mg X) was added 1.20 ml (11.2 mmol) 3-chloroaniline. This mixture was heated one hour at 60°C, then was cooled at room temperature and crystals were precipitated These colourless crystals were washed with ether, the analytical sample was obtained by recrystallisation from mixture ethanol-ether. Yield 58%, mp=213-216°C ^{1}H-NMR (400MHz, DMSO-d_6): 1.40d(6H, J=6.94Hz); 3.48sept (1H, J=6.94Hz); 7.30dd(1H), 7.49dd(1H), 786dd(1H), 8.20s(1H), 8.78s (1H). CHN ($C_{14}H_{14}N_5Cl{\cdot}HCl{\cdot}H_2O$) required: C=49.20%; H=5.01%; N=20.48%; Cl=20.48%; found: C=49.43%; H=5.09%; N=20 13%; Cl=20.58%. MS (ES): $[M+H]^+$=288.5 (100), 290.5 (33).

EXAMPLE 13:

7-(isopent-2-en-(1-yl)amino)-3-isopropylpyrazolo[4,3-d]pyrimidine XIIf

[0051] To chloroform solution of XI (prepared in the subsequent step from 200 mg X) was added 3 5 mL (20 mmol) *N*-ethyldiisopropylamine, 3 mL EtOH and 620 mg (4 7 mmol) isopent-2-en-1-(yl)amino hydrochloride. This mixture was stirred 12 hours at room temperature and then was evaporated to dryness in vacuo. The crude product was purified by column chromatography on silica gel in the mixture of chloroform/methanol/aq. NH_4OH (98:2:1). Yield 48%; syrupy. ^{1}H-NMR (400MHz, $CDCl_3$): 145d(6H, J=6.96Hz); 1.65d(1H, J=1.28Hz), 3.47sept(1H, J=6.96Hz); 4.18d(2H, J=1.28Hz), 5.25m(1H, J=1.28Hz), 6.25s(1H), 8.22s (1H). COSY[1.45d(6H, J=6.96Hz); 3.47sept(1H, J=6.96Hz)], COSY[1.65d(6H, J=1.28Hz); 4.18d(2H, J=1.28Hz); 525m(1H, J=1.28Hz)], COSY[4.18d(2H), 6.25s(1H)]. MS (ES): $[M+H]^+$=246.5 (100).

EXAMPLE 14:

7-furfurylamino-3-isopropylpyrazolo[4,3-d]pyrimidine XIIg

[0052] To the chloroform solution of XI (prepared in the subsequent step from 150 mg X) was added 2 mL (0 205 mol) furfurylamine. This mixture was stirred 1 hour at 50°C and then was evaporated to dryness in vacuo. The crude product was purified by column chromatography on silica gel in the mixture of chloroform/methanol/aq. NH_4OH (98:2: 0.2). Yield 43%; mp=179-182°C ^{1}H-NMR (500MHz, MeOD): 1.422d (6H, J=7.0Hz); 3 455sept (1H, J=7 0Hz); 4 802s (2H), 6 373s (2H); 7 468s (1H); 8.273s (1H). MS (ES): $[M+H]^+$=258.3 (100).

EXAMPLE 15:

7-pentylamino-3-isopropylpyrazolo[4,3-d]pyrimidine XIIh

[0053] To the chloroform solution of XI (prepared in the subsequent step from 150 mg X) was added 0.29 mL (2.53 mmol) 3-pentylamine. This mixture was stirred 1 hour at 50°C and then was evaporated to dryness in vacuo The crude product was purified by column chromatography on silica gel in the mixture of chloroform/methanol (99:1). Yield 25%, mp=73-75°C. ^{1}H-NMR (500MHz, MeOD): 0.933t (3H, J=7.0Hz); 1.374bs (2H); 1.388bs (2H); 1.418d (6H, J=6.9Hz); 1.715pent (2H, J=7.0Hz) 3.447hept (1H, J=6 9Hz), 3.583t (2H, J=7.0Hz); 8.207s (1H). MS (ES): [M+H]$^+$=248.2 (100)

EXAMPLE 16:

7-(2-bromobenzyl)amino-3-isopropylpyrazolo[4,3-d]pyrimidine XIIk

[0054] To chloroform solution of XI (prepared in the subsequent step from 152 mg X, 0.855 mmol) was added 0.44 mL (2.60 mmol) *N*-ethyldiisopropylamine, 2 ml methanol and 343 mg (1.54 mmol) 2-bromobenzylamine hydrochloride. This mixture was heated two hours at 60°C and then was evaporated to dryness in vacuo. The crude product was purified by column chromatography on silica gel in the mixture of chloroform/methanol (98.5:1.5) crystallization from Et_2O Yield 42%, mp=194-196°C. ^{1}H-NMR (300MHz, CH_3OD): 1 44d(6H, J=6.9Hz); 3.43hept(1H, J=6.9Hz); 4.89s(2H); 7.20t(1H, J=7.1Hz); 7.32t(1H, J=7.1Hz); 7.45bs(1H), 7.62d(1H, J=7 1Hz), 8.24s (1H). MS (ES): [M+H]$^+$=246.2 (95), 248.2 (100).

EXAMPLE 17:

7-(4-methoxybenzyl)amino-3-isopropylpyrazolo[4,3-d]pyrimidine XIII

[0055] To chloroform solution of XI (prepared in the subsequent step from 152 mg X, 0.855 mmol) was added 0.34 mL (2.60 mmol) 4-methoxybenzylamine. This mixture was heated one hour at 52°C and then was evaporated to dryness in vacuo The crude product was purified by column chromatography on silica gel in the mixture of chloroform/methanol (98:2), crystallization from Et_2O Yield 42%; mp=143-144°C. ^{1}H-NMR (300MHz, $CDCl_3$): 1 36d(6H, J=6.9Hz); 3.46sept. (1H, J=6.9Hz), 3.69s (3H), 4.86bs (2H); 6.72d (2H, J=8.8Hz); 7.30d (2H, J=8.8Hz); 8.34s (1H). MS (ES): [M+H]$^+$=298.3 (100).

EXAMPLE 18:

7-(3-hydroxy-4-methoxybenzyl)amino-3-isopropylpyrazolo[4,3-d]pyrimidine XIIm

[0056] To chloroform solution of XI (prepared in the subsequent step from 152 mg X, 0.855 mmol) was added 0.44 mL (2.60 mmol) *N*-ethyldiisopropylamine, 7 mL methanol and 236 mg (1.54 mmol) 3-hydroxy-4-methoxybenzylamine. This mixture was heated one hour at 52°C and then was evaporated to dryness in vacuo. The crude product was purified by column chromatography on silica gel in the mixture of chloroform/methanol/NH_4OH (93.5:6.5:0.1) crystallization from chloroform/Et_2O. Yield 62%; mp=197-199°C. ^{1}H-NMR (300MHz, CH_3OD): 1.43d(6H, J=7.15Hz); 4.45hept (1H, J=7.15Hz); 3.83s(3H); 4.67s(2H), 6.82-6.90m (3H), 8.24s (1H). MS (ES): [M+H]$^+$=314.3 (100).

Table 1:

Compounds Prepared by the Method of Examples 8-18					
PYRAZOLO[4,3-d]PYRIMIDINE SUBSTITUENT			CHN ANALYSES	MS ANALYSES-ZMD	
	C7	C3	[%]	[M-H]$^-$ a)	[M+H]$^+$ b)
1	3-chloroanilino	methyl	C=55.50;H=3.88 N=26.97;Cl=13.65		260.1 262. 1
2	anilino	methyl	C=63.99;H=4.92 N=31.09		226.1
3	4-bromoanilino	methyl	C=47 39;H=3.31	303.0	304.0

a) solution: MeOH p.a. + HCOOH
b) solution: MeOH p.a. + H_2O + NH_3

Table 1: (continued)

Compounds Prepared by the Method of Examples 8-18					
PYRAZOLO[4,3-d]PYRIMIDINE SUBSTITUENT			CHN ANALYSES	MS ANALYSES-ZMD	
	C7	C3	[%]	[M-H]- a)	[M+H]+ b)
			N=23.03, Br=26.27	305.0	306 0
4	4-chloroanilino	methyl	C=55.50;H=3.88 N=26 97,Cl=13.65		260.1 262.1
5	2-hydroxybenzylamino	methyl	C=61 17,H=5 13 N=27 43	254.1	256 .1
6	3-hydroxybenzylamimo	methyl	C=61.17;H=5.13 N=27.43	254.1	256.1
7	4- hydroxybenzylamino	methyl	C=61. 17,H=5 13 N=27 43	254.1	256.1
8	2-methylbenzylamino	methyl	C=66.38,H=5 79 N=27.65		254 .1
9	3-methylbenzylamino	methyl	C=66.38;H=5.79 N=27.65		254.1
10	4-methoxybenzylamino	methyl	C=62.44;H=5.61 N=26.00		270.0
11	2-chlorobenzylamino	methyl	C=57.04;H=4.42 N=25.59; Cl=12.95		274.1 276.1
12	2-bromobenzylamino	methyl	C=49.08;H=3.80 N=22.01; Br=25.11		319.0 321.0
13	3-chlorobenzylamino	methyl	C=57.04;H=4.42 N=25.59; Cl=12.95		274.1 276.1
14	3-hydroxy- 4-methoxybenzylamino	methyl	C=58.94;H=5.30 N=24.55		286.0
15	furfurylamino	methyl	C=57.63;H=4.84 N=30.55		230.1
16	allylamino	methyl	C=57.13;H=5.86 N=37.01		190.1
17	cyclohexylamino	methyl	C=62.31;H=7.41 N=30.28		232.2
18	1,4- *(trans)* -cyclohexyldiamino	methyl	C=58.52;H=7.37 N=34.12		247.0
19	1,2-*(cis)*-cyclohexyldiamino	methyl	C=58.52;H=7.37 N=34.12		247.0
20	cyclopentylamino	methyl	C=60.81;H=6.96 N=32.23		218.1
21	cyclobutylamino	methyl	C=59.10;H=6.45 N=34.46		204.1
22	(isopent-2-en-1-yl)amino	methyl	C=60.81;H=6.96 N=32.23		204.1
23	pentylamino	methyl	C=60.25;H=7.81 N=31.94		220.0
24	4-chlorobenzylamino	methyl	C=57.04;H=4.42 N=25.59; Cl=12.95		274.1 276.1

a) solution: MeOH p.a. + HCOOH
b) solution: MeOH p.a. + H_2O + NH_3

Table 2:

Compounds Prepared by the Method of Examples 8-18					
PYRAZOLO[4,3-d]PYRIMIDINE SUBSTITUENT			CHN ANALYSES	MS ANALYSES-ZMD	
	C7	C3	[%]	[M-H]- a)	[M+H]+b)
25	3-chloroanilino	ethyl	C=57.04;H=4.42		274.1

a) solution: MeOH p.a. + HCOOH
b) solution: MeOH p.a. + H_2O + NH_3

Table 2: (continued)

Compounds Prepared by the Method of Examples 8-18					
PYRAZOLO[4,3-d]PYRIMIDINE SUBSTITUENT			CHN ANALYSES	MS ANALYSES-ZMD	
	C7	C3	[%]	[M-H]- a)	[M+H]+b)
			N=25.59;Cl=12.95		276.1
26	anilino	ethyl	C=65.26;H=5.48 N=29.27		240.1
27	4-bromoanilino	ethyl	C=49.07;H=3.80 N=22.01; Br--25.11	317.0 319.0	318.0 320.0
28	4-chloroanilino	ethyl	C=57.04;H=4.42 N=25.59;Cl=12.95		274.1 276.1
29	2-hydroxybenzylamino	ethyl	C=62.44;H-5.61 N=26.00	268.1	270.1
30	3-hydrorrybenzylamino	ethyl	C=62.44;H=5.61 N=26.00	268.1	270.1
31	4- hydroxybenzylamino	ethyl	C=62.44;H=5.61 N=26.00	268.1	270.1
32	2-methylbenzylamino	ethyl	C=67.39;H=6.41 N=26.20		268.2
33	3-methylbenzylamino	ethyl	C=67.39;H=6.41 N=26.20		268.2
34	4- methoxybenzylamino	ethyl	C=63.59;H=6.05 N=24.72		284.0
35	2-chlorobenzylamino	ethyl	C=58.44;H=4.90 N=24.34; Cl=12.32		288.1 290.1
36	2-bromobenzylamino	ethyl	C=50.62;H=4.25 N=21.08; Br=24.05		333.0 335.0
37	3-chlorobenzylamino	ethyl	C=58.44;H=4.90 N=24.34; Cl=12.32		288.1 290.1
38	3-hydroxy-4-methoxybenzylamino	ethyl	C=60.19;H=5.72 N=23.40		300.0
39	furfurylamino	ethyl	C=59.25;H=5.39 N=28.79		244.1
40	allylamino	ethyl	C=59.10;H=6.45 N=34.46		204.1
41	cyclohexylamino	ethyl	C=63.65;H=7.81 N=28.55		246.2
42	1,4-*(trans)*-cyclohexyldiamino	ethyl	C=59.98;H=7.74 N=32.28		261.0
43	1,2-*(cis)*-cyclohexyldiamino	ethyl	C=59.98;H=7.74 N=32.28		261.0
44	cyclopentylamino	ethyl	C=62.31;H=7.41 N=30.28		232.2
45	cyclobutylamino	ethyl	C=60.81;H=6.96 N=32.23		218.1
46	(isopent-2-en-1-yl)amino	ethyl	C=62.31;H=7.41 N=30.28		232.2
47	pentylamino	ethyl	C=61.78;H=8.21 N=30.02		234.0
48	4-chlorobenzylamino	ethyl	C=58.44;H=4.90 N=24.34; Cl=12.32		288.1 290.1

a) solution: MeOH p.a. + HCOOH

b) solution: MeOH p.a. + H_2O + NH_3

Table 3:

Compounds Prepared by the Method of Examples 8-18					
PYRAZOLO[4,3-d]PYRIMIDINE SUBSTITUENT			CHN ANALYSES	MS ANALYSES-ZMD	
	C7	C3	[%]	[M-H]-a)	[M+H]+b)
49	3-chloroanilino	isopropyl	C=58,44;H=4,90 N=24,34;Cl=12,32		288.5 290.5
50	anilino	isopropyl	C=66,38;H=5,97 N=27,65		254.1
51	4-bromoanilino	isopropyl	C=50.62;H=4.25 N=21.08; Br=24.05	331.1 333.1	332.1 334.1
52	4-chloroanilino	isopropyl	C=58,44;H=4,90 N=24,34;Cl=12,32	286.1 288.1	288.1 290.1
53	2-hydroxybenzylamino	isopropyl	C=63.59;H=6.05 N=24.72	272.3	274.3
54	3-hydroxybenzylamino	isopropyl	C=63.59;H=6.05 N=24.72	272.3	274.3
55	4- hydroxybenzylamino	isopropyl	C=63.59;H=6.05 N=24.72	272.3	274.3
56	2-methylbenzylamino	isopropyl	C=68.30;H=6.81 N=24.89		282.2
57	3-methylbenzylamino	isopropyl	C=68.30;H=6.81 N=24.89		282.2
58	4-methoxybenzylamino	isopropyl	C=64.63;H=6.44 N=23.55	296.3	298.3
59	2-chlorobenzylamino	isopropyl	C=59.70;H=5.34 N=23.21; Cl=11.75		302.1 304.1
60	2-bromobenzylamino	isopropyl	C=52.04;H=4.66 N=20.23; Cl=23.08	344.1 346.1	346.1 348.1
61	3-chlorobenzylamino	isopropyl	C=59.70;H=5.34 N=23.21; Cl=11.75		302.1 304.1
62	3-hydroxy-4-methoxybenzylamino	isopropyl	C=61.33;H=6.11 N=22.35	312.3	314.3
63	furfurylamino	isopropyl	C=60.69;H=5.88 N=27.22		258.3
64	allylamino	isopropyl	C=60.81;H=6.96 N=32.23		218.1
65	cyclohexylamino	isopropyl	C=64.84;H=8.16 N=27.00		260.2
66	1,4-*(trans)*-cyclohexyldiamino	isopropyl	C=61.29;H=8.08 N=30.63	273.3	275.3
67	1,2-*(cis)*-cyclohexyldiamino	isopropyl	C=61.29;H=8.08 N=30.63	273.3	275.3
68	cyclopentylamino	isopropyl	C=63.65;H=7.81 N=28 55		246.2
69	cyclobutylamino	isopropyl	C=62.31,H=7.41 N=30.28		232.2
70	isopent-(2-en)-1-ylamimo	isopropyl	C=63.65;H=7.81 N=28.55		246.5
71	pentylamino	isopropyl	C=63 13;H=8.56 N=28.31		248.2
72	4-chlorobenzylamino	isopropyl	C=59.70;H=5.34 N=23.21; Cl=11.75		302.1 304.1

a) solution: MeOH p.a. + HCOOH
b) solution: MeOH p.a. + H_2O + NH_3

EXAMPLE 19: CDK Inhibition Assays

Proteins

[0057] Cyclin-dependent kinases ($P34^{cdc2}$, $p33^{cdk2}$) and cyclins (cyclin B, E) are produced in Sf9 insect cells coin-

fected with appropriate baculoviral constructs. The cells are harvested 68-72 hrs post infection in lysis buffer for 30 min on ice and the soluble fraction is recovered by centrifugation at 14.000 g for 10 min. The protein extract is stored at -80 °C.

[0058] Lysis buffer: 50mM Tris pH 7.4, 150mM NaCl, 5mM EDTA, 20mM NaF, 1% Tween, 1mM DTT, 0.1mM PMSF, leupeptine, aprotonine.

Enzyme inhibition assays

[0059] To carry out experiments on kinetics under linear conditions, the final point test system for kinase activity measurement is used. The kinase is added to reaction mixture in such a way as to obtain linear activity with respect to the concentration of enzyme and with respect to time.

[0060] The $p34^{cdc2}$ and $p33^{cdk2}$ kinase inhibition determination involves the use of 1mg/ml histone H1 (Sigma, type III-S) in the presence of 15 µM [γ-^{33}P]ATP (500-100 cpm/pmol) (ICN) in a final volume of 10 µl. Kinase activity is determined at 30 °C in the kinase buffer.

[0061] Tested compounds are usually dissolved to 100 mM solutions in DMSO, final concentration of DMSO in reaction mixture never exceeds 1%. The controls contain suitable dilutions of DMSO.

[0062] After 10 min, addition 3x SDS sample buffer stops the incubations. Phosphorylated proteins are separated electrophoretically using 10 % SDS polyacrylamide gel. The measurement of kinase activity is done using digital image analysis.

[0063] The kinase activity is expressed as a percentage of maximum activity, the apparent inhibition constants are determined by graphic analysis.

[0064] Kinase buffer: 50 mM Hepes pH 7.4, 10 mM $MgCl_2$, 5 mM EGTA, 10 mM 2-glycerolphosphate, 1 mM NaF, 1 mM DTT

Table 4:

Kinase Inhibitory Activity of Selected 3,7-Disubstituted Pyrazolo[4,3-d]pyrimidine Derivatives			
SUBSTITUENT		CDK1	CDK2
C7	C3	IC_{50} (µM)	IC_{50} (µM)
Olomoucine		7	7
3-chloroanilino	methyl	14	16
anilino	methyl	22	21
3-chloro-5-aminoanilino	methyl	19	24
3-chloro-4-carboxyanilino	methyl	25	28
3-carboxy-4-chloroanilino	methyl	24	29
3-carboxy-4-hydroxyanilino	methyl	34	40
4-bromoanilino	methyl	16	18
4-chloroanilino	methyl	26	28
3-amino-4-chloroanilino	methyl	27	28
3-chloro-4-aminoanilino	methyl	26	28
2-hydroxybenzylamino	methyl	4	5.2
3-hydroxybenzylamino	methyl	5.5	7.2
2-methylbenzylamino	methyl	18	17
3-methylbenzylamino	methyl	28	31
2-chlorobenzylamino	methyl	25	24
3-chlorobenzylamino	methyl	8.8	9.4
furfurylamino	methyl	18	16
allylamino	methyl	42	48

Table 4: (continued)

Kinase Inhibitory Activity of Selected 3,7-Disubstituted Pyrazolo[4.3-d]pyrimidine Derivatives			
SUBSTITUENT		CDK1	CDK2
C7	C3	IC_{50} (μM)	IC_{50} (μM)
cyclohexylamino	methyl	34	32
cyclopentylamino	methyl	29	46
cyclobutylamino	methyl	35	28
isopentenylamino	methyl	45	44
4-chlorobenzylamino	methyl	20	18
benzylamino	ethyl	12	11
3-chloroanilino	ethyl	24	18
anilino	ethyl	14	20
3-chloro-5-aminoanilino	ethyl	20	15.5
3-chloro-4-carboxyanilino	ethyl	36	32
3-carboxy-4-chloroanilino	ethyl	38	40
3-carboxy-4-hydroxyanilino	ethyl	24	22
4-bromoanilino	ethyl	22	18
4-chloroanilino	ethyl	14	16
3-amino-4-chloroanilino	ethyl	23	22
3-chloro-4-aminoanilino	ethyl	35	28
2-hydroxybenzylamino	ethyl	6.2	7
3-hydroxybenzylamino	ethyl	6	3.2
2-methylbenzylamino	ethyl	15	14
3-methylbenzylamino	ethyl	18	17
2-chlorobenzylamino	ethyl	12	12
3-chlorobenzylamino	ethyl	9.7	10.2
furfurylamino	ethyl	8.2	5.3
allylamino	ethyl	25	29
cyclohexylamino	ethyl	39	42
cyclopentylamino	ethyl	32	32
cyclobutylamino	ethyl	27	25
isopentenylamino	ethyl	45	41
4-chlorobenzylamino	ethyl	26	19
benzylamino	isopropyl	1.3	0.5
3-chloroanilino	isopropyl	2.0	0.8
anilino	isopropyl	4.4	5,2
3-chloro-5-aminoanilino	isopropyl	6.1	5.8
3-chloro-4-carboxyanilino	isopropyl	2.5	2.8
3-carboxy-4-chloroanilino	isopropyl	2.8	2.9
3-carboxy-4-hydroxyanilino	isopropyl	3.1	4.2

Table 4: (continued)

Kinase Inhibitory Activity of Selected 3,7-Disubstituted Pyrazolo[4,3-d]pyrimidine Derivatives			
SUBSTITUENT		CDK1	CDK2
C7	C3	IC_{50} (μM)	IC_{50} (μM)
4-bromoanilino	isopropyl	1.7	1.9
4-chloroanilino	isopropyl	2.1	3.1
3-amino-4-chloroanilino	isopropyl	2.9	3.0
3-chloro-4-aminoanilino	isopropyl	2.9	3.0
2-hydroxybenzylamino	isopropyl	0,4	0,27
3-hydroxybenzylamino	isopropyl	1,1	0.9
4-hydroxybenzylamino	isopropyl	1.8	0.2
4-methoxybenzylamino	isopropyl	2.3	1.0
2-methylbenzylamino	isopropyl	2	1.4
3-methylbenzylamino	isopropyl	2.2	3.1
2-chlorobenzylamino	isopropyl	2.1	2.0
3-chlorobenzylamino	isopropyl	8.8	9.4
3-hydroxy-4-methoxy	isopropyl	0,9	0,2
2-bromobenzylamino	isopropyl	7	9
furfurylamino	isopropyl	1,8	0,8
allylamino	isopropyl	16	14
cyclohexylamino	isopropyl	80	95
cyclopentylamino	isopropyl	18	20
(2-aminocyclohexyl)amino	isopropyl	>100	>100
(4-aminocyclohexyl)amino	isopropyl	80	70
pentylamino	isopropyl	1,7	1.4
isopentenylamino	isopropyl	4 5	1.3
4-chlorobenzylamino	isopropyl	1.8	1.1

Table 4 shows the results of inhibitory activity of novel compounds against CDK1 and CDK2 in comparison with the data on a prototype compound (trisubstituted purine olomoucine). Most of the 3,7-disubstituted pyrazolo[4,3-d]pyrimidine derivatives showed marked inhibitory activity in *in vitro* kinase assays

EXAMPLE 20: CDK Inhibitory Activity on Plant Kinases and Antimitotic Effects

[0065] Protein extraction and purification of pant CDK by binding to p13^{suc1}-beads or immunopurification with an antibody specific to the cdc2a-MS protein was carried out as described previously (Bögre et al. 1997, Plant Physiol. 113, 1997, 841-852). The MMK1 protein kinase was purified with a specific antibody from *Vicia faba* extracts as described by Bögre le al. 1997a, Plant Cell 9, 75-83). Protein kinase activity was measured as described above in Example 19. The quantification of radioactivity incorporated into histone H1 or myelin basic protein was undertaken using Phosphoimager (original gel images shown on Fig. 1). IC_{50} were calculated from dose-response curves. The drugs inhibited the activity of immunopurified *Vicia faba* and alfalfa Cdc2-kinase. An observed transient arrest at the G1/S and G2/M indicated that inhibition of the Cdc2-kinase had an effect on both transitions. In contrast to the regular bipolar spindle in untreated cell, in drug-treated metaphase cells abnormally short and dense kinetochore microtubule fibres were observed These microtubules were randomly arranged in the vicinity of the kinetochores and connected the chromosomes. Thus the chromosomes were not aligned on the metaphase plate but were arranged in a circle, with kinetochores pointing inwards and chromosome arms pointing outwards. γ-tubulin, which plays a role in microtubule nucle-

ation, also localised to the centre of the monopolar spindle. The observed abnormalities in mitosis, after inhibition of Cdc2-kinase by specific drugs suggest a role for this enzyme in regulating some of the steps leading to a bipolar spindle structure (Fig. 2 and 3).

Table 5:

Kinase Inhibitory Activity of Selected 3,7-Disubstituted Pyrazolo[4,3-d]pyrimidine Derivatives			
SUBSTITUENT		CDC2a	MMK1
C7	C3	IC_{50} (μM)	IC_{50} (μM)
Olomoucine		**8**	**15.4**
3-chloroanilino	methyl	10	9.6
anilino	methyl	16	14
3-chloro-5-aminoanilino	methyl	21	24
3-chloro-4-carboxyanilino	methyl	27	29
3-carboxy-4-chloroanilino	methyl	25	30
3-carboxy-4-hydroxyanilino	methyl	32	44
4-bromoanilino	methyl	28	28
4-chloroanilino	methyl	26	26
3-amino-4-chloroanilino	methyl	35	34
3-chloro-4-aminoanilino	methyl	30	32
2-hydroxybenzylamino	methyl	4.2	4.8
3-hydroxybenzylamino	methyl	5.8	8.3
2-methylbenzylamino	methyl	20	20
3-methylbenzylamino	methyl	30	31
2-chlorobenzylamino	methyl	25	25
3-chlorobenzylamino	methyl	8.4	10
furfurylamino	methyl	18	16
allylamino	methyl	45	50
Cyclohexylamino	methyl	35	30
Cyclopentylamino	methyl	30	45
Cyclobutylamino	methyl	35	28
Isopentenylamino	methyl	45	44
4-chlorobenzylamino	methyl	20	18
benzylamino	ethyl	14	16
3-chloroanilino	ethyl	22	21
anilino	ethyl	19	24
3-chloro-5-aminoanilino	ethyl	25	28
3-chloro-4-carboxyanilino	ethyl	24	29
3-carboxy-4-chloroanilino	ethyl	34	40
3-carboxy-4-hydroxyanilino	ethyl	16	18
4-bromoanilino	ethyl	26	28
4-chloroanilino	ethyl	27	28

Table 5: (continued)

Kinase Inhibitory Activity of Selected 3,7-Disubstituted Pyrazolo[4.3-d]pyrimidine Derivatives			
SUBSTITUENT		CDC2a	MMK1
C7	C3	IC_{50} (μM)	IC_{50} (μM)
3-amino-4-chloroanilino	ethyl	26	28
3-chloro-4-aminoanilino	ethyl	4	5.2
2-hydroxybenzylamino	ethyl	5.5	7.2
3-hydroxybenzylamino	ethyl	18	17
2-methylbenzylamino	ethyl	28	31
3-methylbenzylamino	ethyl	25	24
2-chlorobenzylamino	ethyl	8.8	9.4
3-chlorobenzylamino	ethyl	18	16
furfurylamino	ethyl	42	48
allylamino	ethyl	34	32
cyclohexylamino	ethyl	29	46
cyclopentylamino	ethyl	35	28
cyclobutylamino	ethyl	45	44
isopentenylamino	ethyl	20	18
4-chlorobenzylamino	ethyl	8	5.3
benzylamino	isopropyl	1.1	1.2
3-chloroanilino	isopropyl	2.0	0.8
anilino	isopropyl	4.4	5.2
3-chloro-5-aminoanilino	isopropyl	2.8	2.9
3-chloro-4-carboxyanilino	isopropyl	3.2	4.1
3-carboxy-4-chloroanilino	isopropyl	1.6	1.7
3-carboxy-4-hydroxyanilino	isopropyl	2.2	3.6
4-bromoanilino	isopropyl	3.2	3.7
4-chloroanilino	isopropyl	3.1	3.0
3-amino-4-chloroanilino	isopropyl	4.1	1.8
3-chloro-4-aminoanilino	isopropyl	0.6	0.4
2-hydroxybenzylamino	isopropyl	1.7	1.3
3-hydroxybenzylamino	isopropyl	2.1	2.7
4-hydroxybenzylamino	isopropyl	2.2	1.8
4-methoxybenzylamino	isopropyl	2.4	3.1
2-methylbenzylamino	isopropyl	1.9	2.2
3-methylbenzylamino	isopropyl	7.4	8.4
2-chlorobenzylamino	isopropyl	1.7	0.7
3-chlorobenzylamino	isopropyl	2.0	1.4
2-bromobenzylamino	isopropyl	5.5	12
furfurylamino	isopropyl	4.3	5.3

Table 5: (continued)

Kinase Inhibitory Activity of Selected 3,7-Disubstituted Pyrazolo[4,3-d]pyrimidine Derivatives			
SUBSTITUENT		CDC2a	MMK1
C7	C3	IC_{50} (μM)	IC_{50} (μM)
allylamino	isopropyl	13.1	15
cyclohexylamino	isopropyl	30	34
cyclopentylamino	isopropyl	20	22
isopentenylamino	isopropyl	14	15
(2-aminocyclohexyl)amino	isopropyl	>100	>100
pentylamino	isopropyl	3.2	4.2
4-chlorobenzylamino	isopropyl	1.7	1.6

Table 5 shows the results of inhibitory activity of novel compounds against plant in comparison with the data on the prototype compounds (disubstituted purines olomoucine, roscovitine and purvalanol A). Most of the 3,7-disubstituted pyrazolo[4,3-d]pyrimidine derivatives showed marked inhibitory activity in *in vitro* plant kinase assays.

EXAMPLE 21: *In vitro* Cytotoxic Activity of Novel Compounds

[0066] We have been using the following cell lines: *HELA* (human cervical carcinoma), *MCF7* (human breast adenocarcinoma), *NIH 3T3* (mouse fibroblasts), *HOS* (human osteogenic sarcoma), *HL 60* (human promyelocytic leukemia), *G 361* (human malignant melanoma), *K562* (human chronic myeloblastic leukemia), *CEM* (human lymphoblastoid leukaemia). Tested drugs were added to the cell cultures in six different concentration and kept at 37 °C and 5% CO_2 for three days. All cell lines were grown in DMEM medium (Gibco BRL) supplemented with 10% (v/v) fetal bovine serum and L-glutamine and maintained at 37 °C in a humidified atmosphere with 5% CO_2. 10^4 cells were seeded into each well of 96 well plate, allowed to stabilize for at least 2 h and then tested compounds were added at various concentrations ranging from 200 to 0,2 μM in triplicates. Three days after drug addition Calcein AM solution (Molecular Probes) was added and let to enter the cells for 1 hour. Fluorescence of viable cells was quantified employing Fluoroskan Ascent (Microsystems). The IC_{50} value, the drug concentration lethal to 50% of the tumour cells, was calculated from the obtained dose response curves (Fig. 6).

[0067] Cytoxicity of novel compounds was tested on panel of cell lines of different histogenetic and species origin (Tab. 6). Higher activities were found in all tumour cell lines tested. Notably, the higher effectiveness of novel derivatives was also found in cell lines bearing various mutations or deletions in cell cycle associated proteins, e.g. HL-60, BT549, Hela, U2OS, MDA-MB231, and Saos2. It indicates that these substances should be equally effective in tumours with various alterations of tumour suppressor genes, namely p53, Rb, etc. Importantly, this observation distinguishes the novel compounds from flavopiridol and related compounds, as their biological activity is dependent on p53 status.

Table 6:

Cytotoxicity of Novel Compounds for Different Cancer Cell Lines			
SUBSTITUENT		MCF7	K-562
C7	C3	IC_{50} (μM)	IC_{50} (μM)
Olomoucine		131,8	>167
benzylamino	methyl	67	89
3-chloroanilino	methyl	24	35
2-hydroxybenzylamino	methyl	67	55
3-hydroxybenzylamino	methyl	119	143
4-hydroxybenzylamino	methyl	>167	>167
3-hydroxy-4-methoxybenzylamino	methyl	58	79
4-methoxybenzylamino	methyl	35	53

Table 6: (continued)

Cytotoxicity of Novel Compounds for Different Cancer Cell Lines			
SUBSTITUENT		MCF7	K-562
C7	C3	IC_{50} (μM)	IC_{50} (μM)
furfurylamino	methyl	142	>167
pentylamino	methyl	45	67
cyclobutylamino	methyl	89	101
4-aminocyclohexylamino	methyl	75	115
2-bromobenzylamino	methyl	56	68
benzylamino	ethyl	62	81
3-chloroanilino	ethyl	19	28
2-hydroxybenzylamino	ethyl	63	51
3-hydroxybenzylamino	ethyl	111	132
3-hydroxy-4-methoxybenzylamino	ethyl	52	72
4-methoxybenzylamino	ethyl	31	48
furfurylamino	ethyl	135	>167
pentylamino	ethyl	41	62
cyclobutylamino	ethyl	82	94
4-aminocyclohexylamino	ethyl	71	110
2-bromobenzylamino	ethyl	54	60
benzylamino	isopropyl	55	72
3-chloroanilino	isopropyl	9	12
anilino	isopropyl	15	21
3-chloro-5-aminoanilino	isopropyl	29	35
3-chloro-4-carboxyanilino	isopropyl	46	69
3-carboxy-4-chloroanilino	isopropyl	0.4	1
3-carboxy-4-hydroxyanilino	isopropyl	12	25
4-bromoanilino	isopropyl	5	7
4-chloroanilino	isopropyl	3	4
3-amino-4-chloroanilino	isopropyl	0..2	0.3
3-chloro-4-aminoanilino	isopropyl	12	13
2-hydroxybenzylamino	isopropyl	63	50
3-hydroxybenzylamino	isopropyl	105	132
4-hydroxybenzylamino	isopropyl	152	>167
3-hydroxy-4-methoxybenzylamino	isopropyl	45	68
4-methoxybenzylamino	isopropyl	28	41
2-methylbenzylamino	isopropyl	63	75
3-methylbenyzylamino	isopropyl	76	94
2-chlorobenzylamino	isopropyl	15	21
3-chlorobenzylamino	isopropyl	24	26

Table 6: (continued)

Cytotoxicity of Novel Compounds for Different Cancer Cell Lines			
SUBSTITUENT		MCF7	K-562
C7	C3	IC_{50} (μM)	IC_{50} (μM)
furfurylamino	isopropyl	130	>167
allylamino	isopropyl	64	77
cyclohexylamino	isopropyl	95	98
pentylamino	isopropyl	32	61
cyclobutylamino	isopropyl	45	60
isopentenylamino	isopropyl	>167	>167
2-aminocyclohexylamino	isopropyl	> 167	> 167
4-aminocyclohexylamino	isopropyl	68	107
2-bromobenzylamino	isopropyl	42	57
2-hydroxy-3 -methoxybenzylamino	isopropyl	0.6	0 8
2-hydroxy-4-methoxybenzylamino	isopropyl	0 2	0.5
2-hydroxy-5-methoxybenzylamino	isopropyl	0.9	1.2
2-aminobenzylamino	isopropyl	0.8	2.1

EXAMPLE 9: Novel Compounds Have Cytotoxic Effects for Plant Cells and Induce their Apoptosis

[0068] The novel compounds have also been tested in tobacco callus bioassay for cytotoxicity (herbicidal activity) and induction of cell death. The compounds to be tested were dissolved in dimethylsulfoxide (DMSO) and the solution brought up to 10^{-3} M with distilled water. This tock solution was further diluted in the respective media used for the tobacco bioassay to concentration ranging from 10^{-8} M to 10^{-4} M. The final concentration of DMSO in the media did not exceed 0.2%, and therefore did not affect biological activity in the assay system used. Cytokinin-dependent tobacco callus *Nicotiana tabacum* L. cv. Wisconsins 38 Murashige-Skoog medium, containing per 1 liter: 4μmol nicotinic acid, 2.4 μmol pyridoxine hydrochloride, 1.2 μmol thiamine, 26.6 μmol glycine, 1.37 μmol glutamine, 1.8 μmol myoinositol, 30 g of sucrose, 8 g of agar, 5.37 μmol α-naphtylacetic acid and 0.5 μmol 6-benzylaminopurine. Subcultivation was carried out every three weeks. Fourteen days before the bioassay, the callus tissue was transferred to the media without 6-benzylaminopurine. Compounds were tested with two different concentrations of 6-benzylaminopurine (10^{-5} M and 10^{-6} M). Inhibitory growth activity was determined from the increase in fresh callus weight after four weeks of cultivation. Five replicates were prepared for each concentration tested and the entire test was repeated at least twice. Inhibitory activity was compared with growth response curve of 6-benzylaminopurine in the range from 10^{-8} M to 10^{-4} M and IC50 was calculated for each compound for 10^{-5} M and 10^{-6} M of 6-benzylaminopurine.

Table 7:

Cytotoxicity of Novel Compounds for Tobacco Plant Cells Cultivated *in vitro*.			
SUBSTITUENT		10^{-5} M BAP	10^{-6} M BAP
C7	C3	IC_{50} (μM)	IC_{50} (μM)
OLOMOUCIN		>50	>50
benzylamino	isopropyl	24	6.5
3-chloroanilino	isopropyl	34	8.2
anilino	isopropyl	41	5.3
2-methylbenzylamino	isopropyl	14	4.2
3-methylbenzylamino	isopropyl	26	8 5

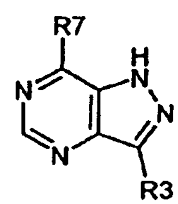
Table 7: (continued)

Cytotoxicity of Novel Compounds for Tobacco Plant Cells Cultivated *in vitro*			
SUBSTITUENT		10^{-5} M BAP	10^{-6} M BAP
C7	C3	IC_{50} (µM)	IC_{50} (µM)
2-chlorobenzylamino	isopropyl	31	4.6
3-chlorobenzylamino	isopropyl	20	1.7
furfurylamino	isopropyl	25	9.4
allylamino	isopropyl	18	3.7
cyclohexylamino	isopropyl	46	9 4
cyclopentylamino	isopropyl	15.6	8.4
cyclobutylamino	isopropyl	12.1	4.3
isopentenylamino	isopropyl	15.3	1.7
4-chlorobenzylamino	isopropyl	12.1	4.7

[0069] Table 7 shows the results of inhibitory activity of novel compounds on growth of tobacco cells cultivated *in vitro* in comparison with the data on the prototype compound (trisubstituted purine olomoucine). Most of the 3,7-disubstituted pyrazolo[4,3-d]pyrimidine derivatives showed marked inhibitory activity on *in vitro* growth. Furthermore, these compounds are able to induce apoptosis in plants cells (are able to kill plant cells) and induce strong antimitotic activities (see Fig.4 and 5). A dose-dependent inhibition of the cell cycle in G1/S and G2/M transition points was observed. The appearance of DNA fragmentation observed by DNA double strand breaks labelling in situ started 3h after drugs addition with highest frequency after 24-48h of treatment, when oligonucleosomal DNA ladder occurred. The high doses of roscovitine, bohemine which induced apoptosis were shown to downregulate in vivo activity of cdk; decrease of cdk protein level was shown by Western blotting and immunofluorescence labelling. Microtubule reorganization contributing to apoptotic morphology.was observed. The results presented here clearly show that the novel compounds exhibit herbicidal activity.

Claims

1. 3-, 7-disubstituted pyrazolo[4,3-d]pyrimidines represented by the general formula I



I

and pharmaceutically acceptable salts thereof, wherein
R3 is selected from the group consisting of
alkyl, cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl alkyl, cycloheteroalkyl, aryl, heterocycle, heteroaryl, arylalkyl, heteroarylalkyl, and heteroalkyl, wherein each of the groups may optionally be substituted,
R7 is selected from the group consisting of halogen, hydroxyl, hydroxylamino, amino, carboxyl, cyano, nitro, amido, sulfo, sulfamido, carbamino, unsubstituted or substituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted arylalkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroarylakyl, substituted or unsubstituted cycloalkyl alkyl, substituted or unsubstituted cycloheteroalkyl alkyl;

R7'-X- wherein X is an -NH-, -N(alkyl)-, -O- or -S- moiety and R7' is selected from the group consisting ofH, alkyl, cycloalkyl, aryl, alkylcycloalkyl, arylalkyl, heterocycle, heterocycloalkyl, substituted alkyl, substituted cycloalkyl, substituted aryl, substituted arylalkyl, substituted heterocycle, substituted heteroaryl, substituted heteroarylalkyl, substituted heteroalkyl, substituted cycloalkyl alkyl and substituted cycloheteroalkyl alkyl.

2. 3-, 7-disubstituted pyrazolo[4,3-d]pyrimidines according to claim 1, wherein the groups are substituted by more than one halogen, hydroxyl, amino, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, carbamino, alkyl, alkoxy, and/or substituted alkyl group.

3. 3-, 7-disubstituted pyrazolo[4,3-d]pyrimidines according to claims 1 or 2, wherein
R3 is selected from the group consisting of
alkyl, cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, cycloheteroalkylalkyl, cycloalkyl alkyl, aryl, arylakyl, heteroaryl, heteroarylalkyl, wherein each of the groups may be optionally be subsituted by 1-3 halogens like fluoro and chloro;
R7 is selected from the group consisting of halogen, hydroxyl, hydroxylamino, amino, hydrazino, carboxyl, cyano, nitro, amido, sulfo, sulfamido, carbamino, NHCONH2, NHC(=NH)NH2, alkyl, cycloalkyl, cycloalkylalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, cycloheteroalky, cycloheteroalkylalkyl, which is substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, acylamino, acyloxy, alkylamino, dialkylamino, alkylthio and carbamoyl group;
R7'-X, wherein X is -NH-, -O-, -S-; or -N(alkyl)- and
R7'-X, wherein X is preferably -N(alkyl)- selected at each occurence from the group methyl, ethyl, propyl, isoproyl, ethinyl, ally, propargyl, isopent.2-en-1-yl; R7' is
C_1-C_8 branched or unbranched alkyl, alkenyl or alkinyl selected from the group consisting of methyl, ethyl, isopropyl, butyl, isobutyl, allyl, propargyl, isopent-2en-1-yl, and 2-methylallyl, which are substituted independently at each occurrence with 0 - 5 substituents selected from the group consisting of halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, acylamino, acyloxy, alkylamino, dialkylamino, alkylthio and carbamoyl group; acyl, -C(O)R_a, wherein R_a is C_1-C_6 branched or unbranched alkyl, alkenyl or alkinyl selected from the group consisting of methyl, ethyl, isopropyl, butyl, isobutyl, allyl, propargyl, isopent-2en-1-yl, and 2-methylallyl, which are substituted independently at each occurrence with 0 - 5 substituents selected from the group consisting of halogen, hydroxy, alkoxy, amino, hydrazo, mercapto, carboxyl, cyano, nitro, amido, sulfo, sulfamido, acylamino, acyloxy, alkylamino, dialkylamino, alkylthio and carbamoyl group;
amido, -C(O)N$R_b$$R_c$, wherein R_b and R_c is C_1-C_6 branched or unbranched alkyl, alkenyl or alkinyl selected from the group defined above for C_1-C_6 branched or unbranched alkyl, which is substituted independently at each occurrence with 0 - 5 substituents selected from the group defined above for acyl;
sulfo, -SO_3R_d, wherein R_d is C_1-C_6 branched or unbranched alkyl, alkenyl or alkinyl selected from the group consisting of methyl, ethyl, isopropyl, butyl, isobutyl, , allyl, propargyl, isopent-2en-1-yl, and 2-methylallyl, which is substituted independently at each occurrence with 0 - 5 substituents selected from the group defined above for acyl;
C_3-C_{15} cycloalkyl selected from the group consisting of cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl or adamantyl;
substituted C_3-C_{15} cycloalkyl selected from the group consisting of cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl or adamantyl substituted independently at each occurrence with 0 - 5 substituents selected from the group defined above for acyl;
R_f(cycloalkyl), wherein R_f is
C_1-C_6 alkyl, alkenyl or alkinyl group defined above
C_3-C_{15} cycloalkyl is selected from the group consisting of cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl or adamantyl;
substituted C_3-C_{15} cycloalkyl selected from the group consisting of cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl or adamantyl substituted independently at each occurrence with 0 - 5 substituents selected from the group defined above for acyl;
aryl is selected from the group consisting of phenyl, biphenyl, naphthyl, tetrahydronaphtyl, fluorenyl, indenyl or fenanthrenyl substituted independently at each occurrence with 0 - 5 substituents selected from the group defined above for acyl;
heterocycle is selected from the group consisting of thienyl, furyl, pyranyl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, isothiazolyl, isoxazyl substituted independently at each occurrence with 0 - 5 substituents selected from the group defined above for acyl;
heteroalkyl is -Rg-Het, wherein
R_g is C_1-C_6 alkyl, alkenyl or alkinyl selected from the group methylen, 1,2-ethyliden, 1,3-propiliden, 1,4-butyliden,

pentamethylen, hexamethylen, ethylendiyl, allyl-1,3-diyl, methylethan-1,1-diyl, methylethan-1,2-diyl, butan-1,3-diyl, which is substituted independently at each occurrence with 0 - 5 substituents selected from the group halogen, hydroxy, alkoxy, cyano;
Het is heterocycle as defined above;
heteroaryl is -R_h-HetAr, wherein
R_h is is C_1-C_6 alkyl, alkenyl or alkinyl selected from the group above, and
HetAr is selected from the group consisting of benzothienyl, naphthothienyl, benzofuranyl, chromenyl, indolyl, isoindolyl, indazolyl, qinolyl, isoqinolyl, ftalazinyl, qinaxalinyl, cinnolinyl, qinazolinyl substituted independently at each occurrence with 0 - 5 substituents selected from the group defined above for acyl; arylalkyl is -R_iAr, wherein
R_i is C_1-C_6 alkyl, alkenyl or alkinyl defined above and
Ar is aryl as defined above;
cycloheteroalkyl is selected from the group consisting of piperidinyl, piperazinyl, morfolinyl, pyrrolidinyl, imidazolidinyl substituted independently at each occurrence with 0 - 5 substituents selected from the group defined above for acyl; cycloheteroalkyl alkyl, -R_j(cycloheteroalkyl), wherein
R_j is arylalkyl -R_iAr, wherein
R_i is C_1-C_6 alkyl, alkenyl or alkinyl defined above, and
Ar is aryl as defined above, and
cycloheteroalkyl is selected from the group consisting of piperidinyl, piperazinyl, morfolinyl, pyrrolidinyl, imidazolidinyl substituted independently at each occurrence with 0 - 5 substituents selected from the group defined above for acyl; heteroarylalkyl is -R_k-HetAr, wherein
R_k is C_1-C_6 alkyl, alkenyl or alkinyl as defined above, and
HetAr is heteroaryl group as defined above.

4. 3-, 7-disubstituted pyrazolo[4,3-d]pyrimidines according to any of the preceding claims, which has independently at each occurrence (R) or (S) configuration in R3 or R7 in case of their chirality.

5. A method of preparing the 3-,7-disubstituted pyrazolo[4,3-d]pyrimidine of the formula I, according to claim 1 wherein R7 and R3 substituents are as defined in claim 1, wherein 3-Substituted-7-hydroxypyrazolo[4,3-d]pyrimidines are chlorinated, preferably with $SOCl_2$ or $POCl_3$ or PCl_5, or 7-hydroxy group is displaced by mercapto group, preferably by action of P_2S_5,
the thus obtained 7-chloro-3-substituted pyrazolo[4,3-d]pyrimidines are then reacted with an appropriate reactant nucleophil, or 7-mercapto-3-substituted pyrazolo[4,3-d]pyrimidines are reacted with an alkylating agent, and the resulting 3,7-disubstituted pyrazolo[4,3-d]pyrimidine is optionally isolated.

6. The method of claim 5, wherein the appropriate nucleophil R7'-X-Y is selected from the group consisting of 1) amine (amonium hydroxide, hydrazine, hydroxylamine, benzylamine; 2-, 3-, 4-hydroxybenzylamine; dihydroxybenzylamine; and 3-chloroaniline, or 2) lithium, natrium, and kalium salt of alkohole or mercaptane.

7. The medod of claims 5 or 6, wherein the alkylating agent ist selected from the group consisting of alkylhalogenide, alkyltoluensulfonate, and alkylmesylate.

8. The method of claims 5 to 7, wherein the the resulting 3,7-disubstituted pyrazolo[4,3-d]pyrimidine is isolated by chromatography on silica gel followed by crystallization or only by crystalization.

9. A method for inhibiting cell proliferation in mammals comprising administering effective amount of a compound according to any one of claims 1 to 4 or a pharmaceutically acceptable salt of such a compound together with a pharmaceutical carrier.

10. A method of treating cancer, or psoriasis, rheumatoid arthritis, lupus, type I diabetes, multiple sclerosis, restenosis, polycystic kidney disease, graft rejection, graft versus host disease and gout, parasitoses such as those caused by fungi or protists, or Alzheimer's disease, or as antineurogenerative drugs, or to suppress immunostimulation comprising administering effective amount of 3-, 7-disubstituted pyrazolo[4,3-d]pyrimidines according to any one of claims 1 to 4 or a pharmaceutically acceptable salt thereof together with a pharmaceutical carrier.

11. A method of treating cancer comprising administering effective amount 3-, 7-disubstituted pyrazolo[4,3-d]pyrimidines according to any one of claims 1 to 4 or a pharmaceutically acceptable salt thereof in combination with usually used cytostatics, such as mitoxantrone, cis-platinum, methotrexate, taxol, or doxorubicin.

12. A method of treating an hyperproliferative skin disease in a human suffering therefrom by actinic keratosis, Bowen'sdisease, papilloma, seborrheic keratosis, toxic eczema, atopic dermatitis and ichthyosis comprising administering to a subject a therapeutically effective amount of 3-, 7-diisubstituted pyrazolo[4,3-d]pyrimidines according to any one of claims 1 to 4 or a pharmaceutically acceptable salt thereof together with a pharmaceutical carrier.

13. A method of treating viral infections comprising administering to a subject a therapeutically effective amount of 3-, 7-disubstituted pyrazolo[4,3-d]pyrimidines according to any one of claims 1 to 4 or a pharmaceutically acceptable salt thereof together with a pharmaceutical carrier.

14. 3-, 7-disubstituted pyrazolo[4,3-d]pyrimidines according to any one of claims 1 to 4 as a therapeutic agent.

15. Use of 3-, 7-disubstituted pyrazolo[4,3-d]pyrimidines according to claims 1 to 4 in the preparation of a medicament for the treatment of cancer, or psoriasis, rheumatoid arthritis, lupus, type I diabetes, multiple sclerosis, restenosis, polycystic kidney disease, graft rejection, graft versus host disease and gout, parasitoses such as those caused by fungi or protists, or Alzheimer's disease, asthma, actinic keratosis, Bowen'sdisease, papilloma, seborrheic keratosis, toxic eczema, atopic dermatitis and ichthyosis, cardiovascular, neurodegenerative, viral and inflammatory diseases.

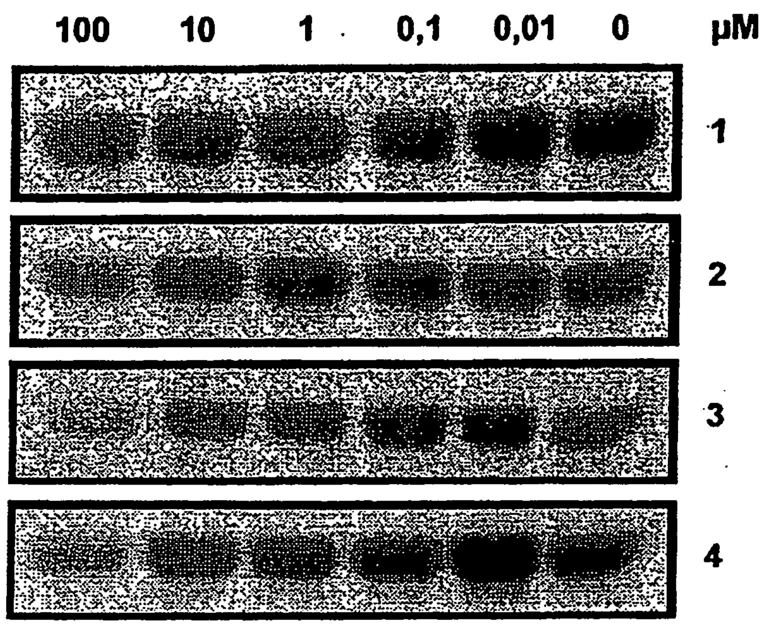


Fig. 1

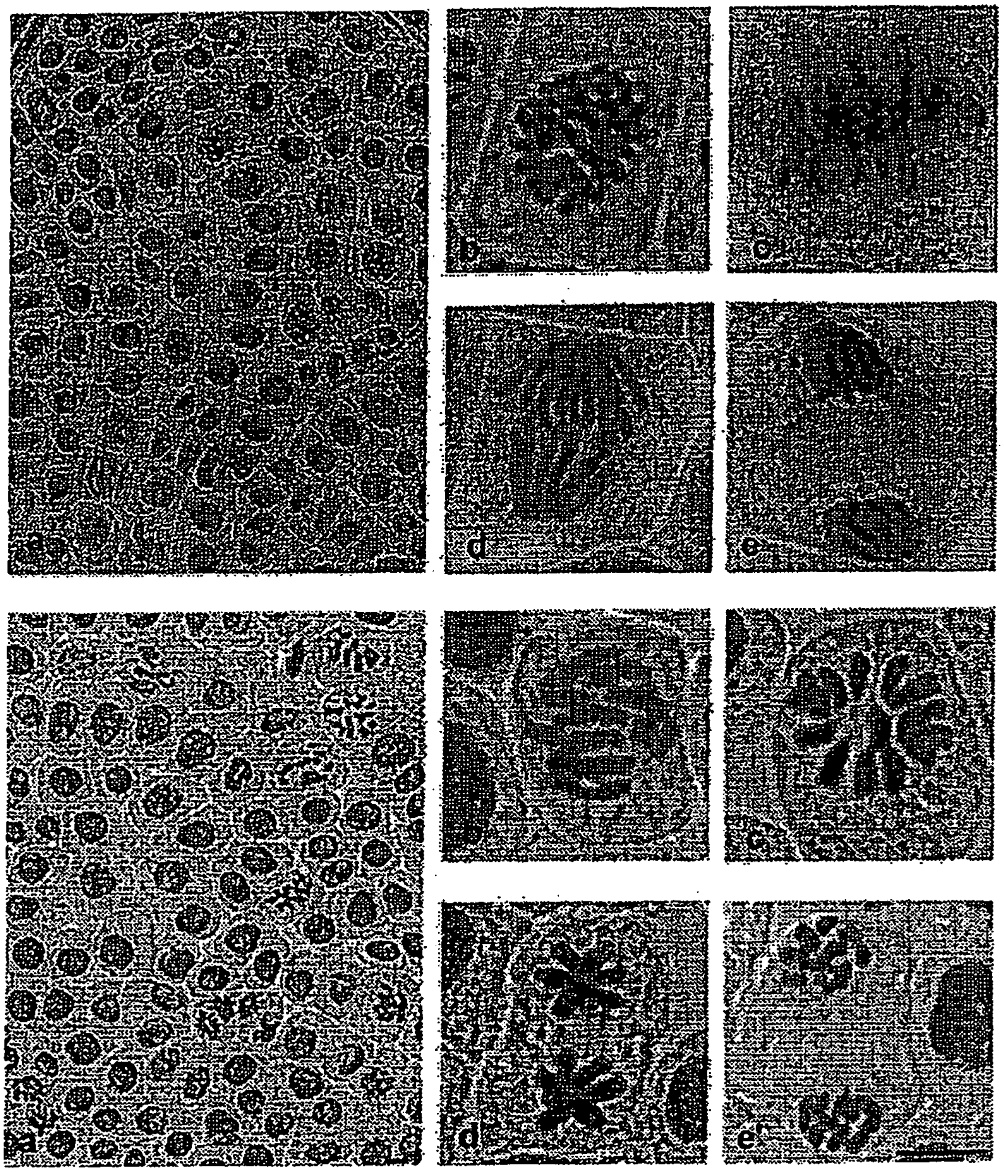


Fig. 2

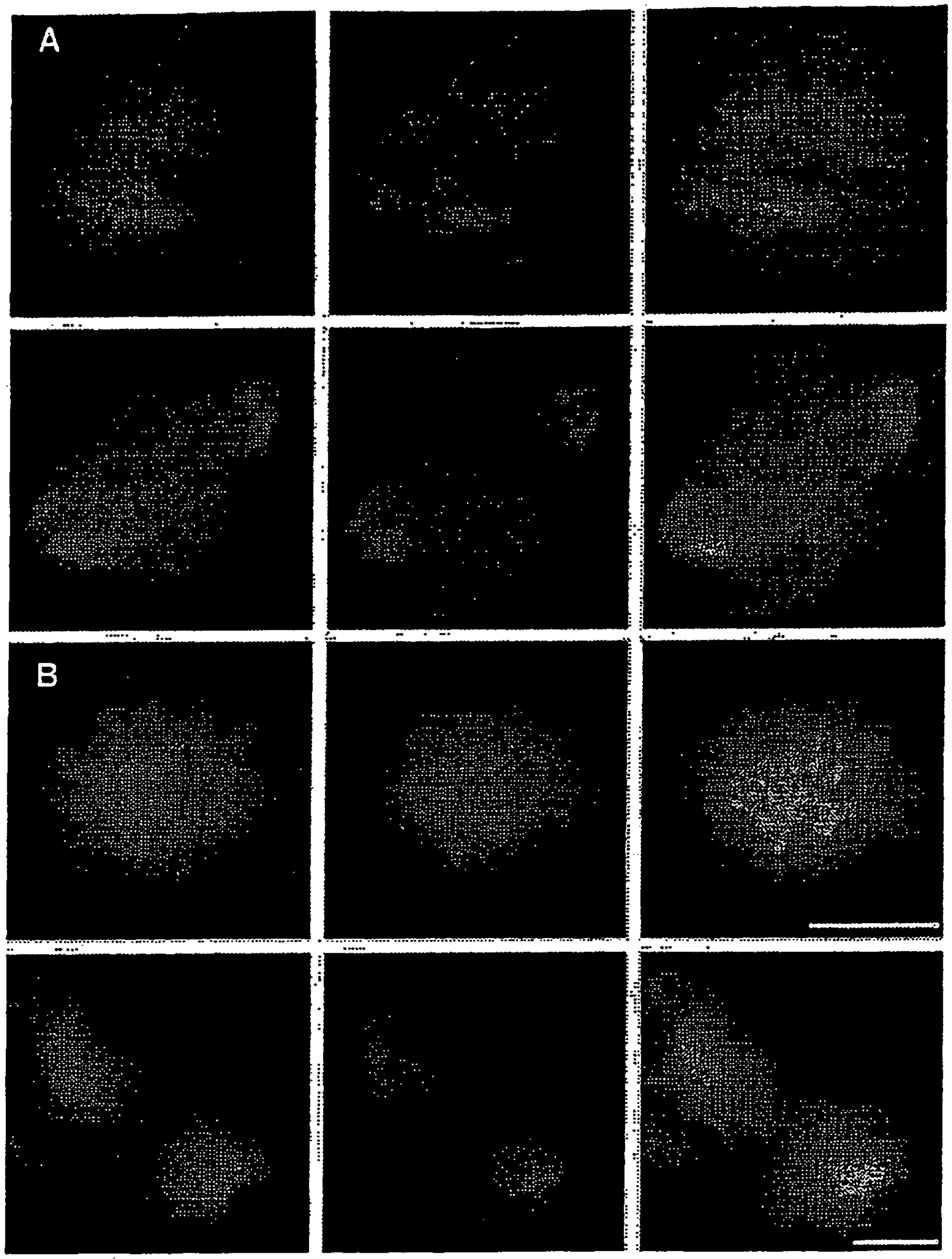


Fig. 3

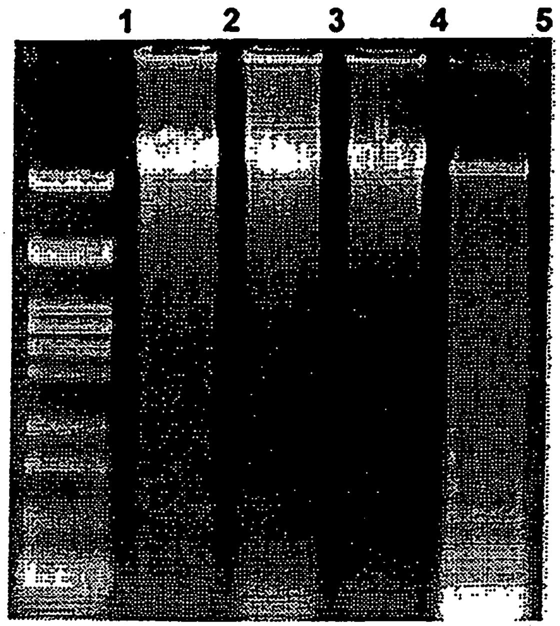


Fig. 4

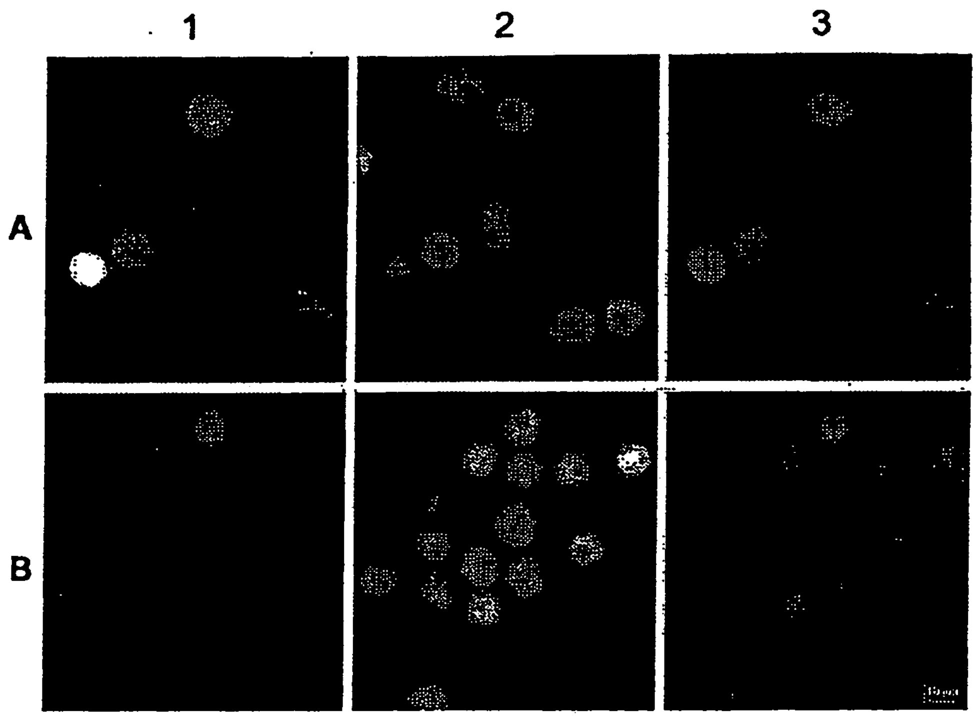


Fig. 5

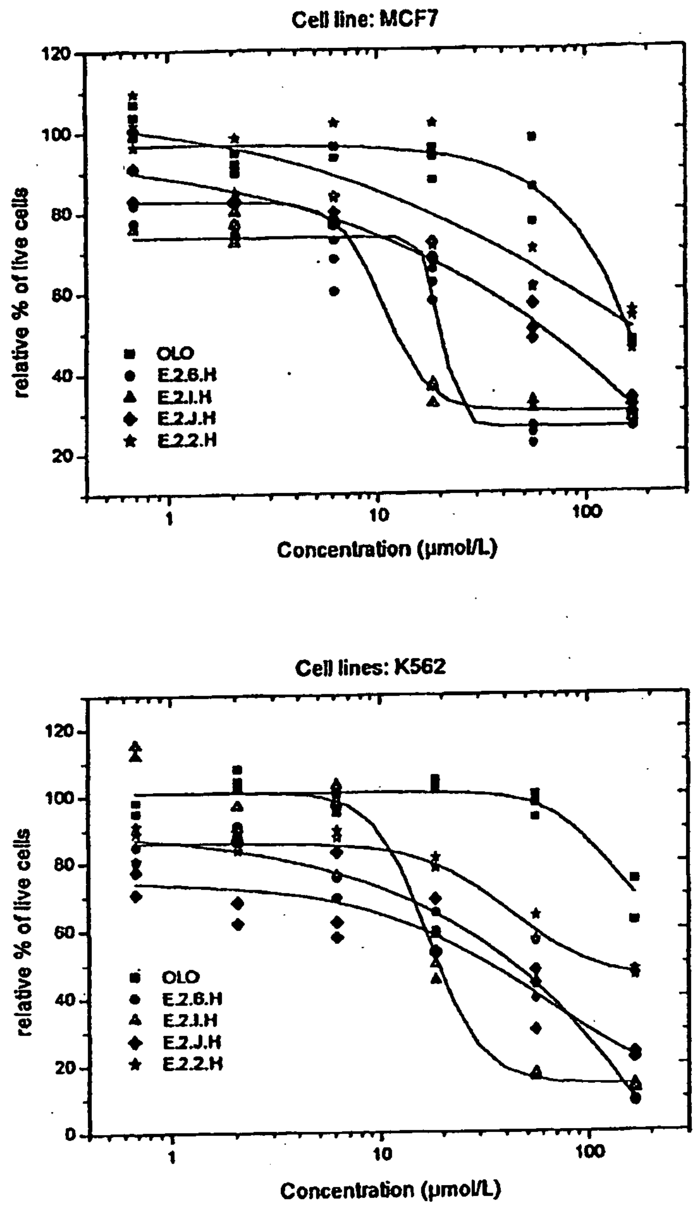

Cell line: MCF7
120
100
80
60
40
20
relative % of live cells
1
10
100
Concentration (μmol/L)
OLO
E.2.6.H
E.2.I.H
E.2.J.H
E.2.2.H
Cell lines: K562
120
100
80
60
40
20
0
relative % of live cells
1
10
100
Concentration (μmol/L)
OLO
E.2.6.H
E.2.I.H
E.2.J.H
E.2.2.H

Fig. 6

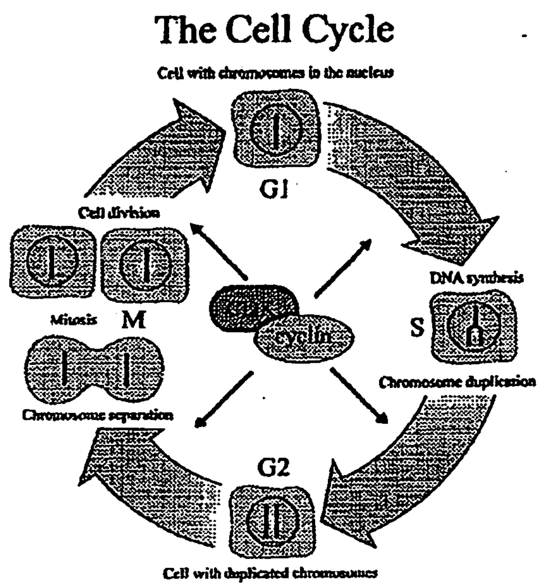

The Cell Cycle
Cell with chromosomes in the nucleus
G1
DNA synthesis
S
Chromosome duplication
G2
Cell with duplicated chromosomes
Chromosome separation
Mitosis
M
Cell division
cyclin

Fig. 7

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PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number
EP 03 01 0184

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	ELLAMES G J ET AL: "THE SYNTHESES OF ACYCLOFORMYCINS AND 5-AMINO-3-(2-HYDROXYETHOXY)-METHYLPYRAZOLO[4,3-D]PYRIMIDIN-7(6H)- ONE AN ANALOGUE OF THE ANTIVIRAL ACYCLOGUANOSINE" JOURNAL OF THE CHEMICAL SOCIETY, PERKIN TRANSACTIONS 1, CHEMICAL SOCIETY. LETCHWORTH, GB, no. 10, 1 October 1985 (1985-10-01), pages 2087-2091, XP002047056 ISSN: 0300-922X compounds 1 and 7 * page 2087, column 1, line 1-3 * * page 2090, column 2, paragraph 4 *	1-4	A61K31/505 C07D487/04 //(C07D487/04, 239:00,231:00)
X	US 1 530 747 A (WYATT FRANK B) 24 March 1925 (1925-03-24) * page 3, line 16 - line 20; table 1 *	1-4	
X	WO 01 18170 A (LOOKEREN CAMPAGNE MICHIEL MARI ;KRIEKEN WILHELMUS MARIA V D (NL);) 15 March 2001 (2001-03-15) * page 5, line 34 - page 6, line 4; claim 1 *	1-4	TECHNICAL FIELDS SEARCHED (Int.Cl.7) C07D A61K

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INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
MUNICH	28 August 2003	Bakboord, J

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C07)

European Patent Office

INCOMPLETE SEARCH SHEET C

Application Number
EP 03 01 0184

Although claims 9-13 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

Claim(s) searched incompletely:
1-15

Reason for the limitation of the search:

The initial phase of the search revealed a very large number of documents relevant to the issue of novelty. So many documents were retrieved that it is impossible to determine which parts of the claims may be said to define subject-matter for which protection might legitimately be sought (Article 84 EPC). For these reasons, a meaningful search over the whole breadth of the claims is impossible. Consequently, the search has been restricted to:

compounds of formula I in which R3 is alkyl and R7 is R7'-X wherein X is an NH moiety as the examples seem to be limited to these compounds.

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 03 01 0184

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	LONG ROBERT A ET AL: "Derivatives of the new ring system pyrazolo[4,3-d]-v-triazine and the synthesis of 5,7-disubstituted 3-methylpyrazolo[4,3- d]pyrimidines and 5,7-disubstituted 3-methylpyrazolo[4,3-d]pyrimidine 6-oxides which are structurally related to the nucleoside antibiotics formycin and formycin B" JOURNAL OF HETEROCYCLIC CHEMISTRY, HETEROCORPORATION. PROVO, US, vol. 7, no. 4, 1970, pages 863-869, XP002208252 ISSN: 0022-152X * example 20 * ---	1-4	
X	US 4 282 361 A (HECHT SIDNEY M ET AL) 4 August 1981 (1981-08-04) * example 11 * ---	1-4	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
X	HECHT SM ET AL: "cytokinins: development of a potent antagonist" PROCEEDING OF THE NATIONAL ACADEMIE OF SCIENCE USA, vol. 68, no. 10, 1971, pages 2608-2610, XP009015448 USA compound 6 * page 2610 * ---	1-4	
X	HECHT SM ET AL: "question of the ribosyl moiety in the promotion of callus growth by exogenously added cytokinins" BIOCHEMISTRY, vol. 10, no. 23, 1971, pages 4224-4228, XP002250881 USA * page 4277, column 2; example 11 * ---	1-4	

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European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 03 01 0184

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	HECHT SM ET AL: "on the activation of cytokinins" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 250, no. 18, 1975, pages 7343-7351, XP002250882 USA * page 7348, line 32 - line 37; examples 18-20 * ---	1-4	
X	SKOOG F ET AL: "cytokinin antagonists: synthesis and physiological effects of 7-substituted 3-methylpyrazolo[4,3-d]pyrimidines" PHYTOCHEMISTRY, vol. 12, 1973, pages 25-37, XP009015452 UK * examples II-XII * ---	1-4	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
X	GREGORINI G ET AL: "biological effects of cytokinin antagonists 7-(pentylamino) and 7-(benzylamino)-3-methylpyrazolo[4,3-d]pyrimidines on suspension-cultured tobacco cells" PLANT PHYSIOLOGY, vol. 65, 1980, pages 363-367, XP001154399 * page 366; examples I,,II * ---	1-4	
X	HECHT SM ET AL: "competitive inhibition of beef heart cyclic AMP phosphodiesterase by cytokinins and related compounds" PROCEEDING OF THE NATIONAL ACADEMIE OF SCIENCES USA, vol. 71, no. 12, 1974, pages 4670-4674, XP009015447 USA * examples 7-11 * ---	1-4	
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EPO FORM 1503 03.82 (P04C10)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 03 01 0184

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
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X	DIZENGREMEL P ET AL: "inhibition by adenine derivatives of the cyaninde insensitive electron transport pathway of plant mitochondria" PLANT PHYSIOLOGY, vol. 70, 1982, pages 585-589, XP001154398 * the whole document *	1-4	
X	BIANCO-COLOMANS J : "effect of a cytokinin antagonist on cytokinin and light-dependent amaranthin synthesis in amaranthus tricolor seedlings" JOURNAL OF PLANT GROWTH REGULATION, vol. 2, no. 4, 1984, pages 281-287, XP009015443 Germany * the whole document *	1-4	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
X	PARKER CW ET AL: "inhibitors of two enzymes which metabolize cytokinins" PHYTOCHEMISTRY, vol. 25, no. 2, 1986, pages 303-310, XP001154032 GB * page 307; example 13 *	1-4	
X	AUNG LH: "Action of cytokinins and anticytokinins on cotyledonary bud growth of lycopersicon esculentum mill" BIOLOGIA PLANTARUM, vol. 28, no. 6, 1986, pages 407-411, XP009015451 praha * the whole document *	1-4	

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PARTIAL EUROPEAN SEARCH REPORT

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EP 03 01 0184

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	WIERZCHOWSKI J ET AL: "luminiscence studies on formycin, its aglycone, and thier N-methyl derivatives: tautomerism, sites of protonation and phototautomerism" PHOTOCHEMISTRY AND PHOTOBIOLOGY, vol. 35, 1982, pages 445-458, XP009015440 GB * table 2 *	1-4	
X	ROBINS RK ET AL: "potential purine antagonists V. synthesis of some 3-methyl-5,7-substituted pyrazolo[4,3-d]pyrimidines" THE JOURNAL OF ORGANIC CHEMISTRY, vol. 21, no. 8, 1956, pages 833-836, XP002250883 USA * table 1 *	1-4	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
X	WIERZCHOWSKI J ET AL: "analogues of formycins A and B: synthesis and some properties of methyl derivatives of 7-amino and 7-keto pyrazolo[4,3-d]pyrimidines" ACTA BIOCHIMICA POLONICA, vol. 27, no. 1, 1980, pages 35-56, XP009015441 Poland * page 50; examples XII,XIII,XIV *	1-4	

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EPO FORM 1503 03.82 (P04C10)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 03 01 0184

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
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X	BUCHANAN JG ET AL: "C-nucleoside studies part 18 the synthesis of C-nucleoside analogues of the antiviral agent (S)-9-(2,3-dihydroxypropyl)adenine" JOURNAL OF THE CHEMICAL SOCIETY PERKIN TRANSACTIONS I, 1985, pages 1425-1430, XP009015442 GB * page 1429, column 2; examples 6,31 * ---	1-4	
A	DE 100 60 388 A (MERCK PATENT GMBH) 6 June 2002 (2002-06-06) * claim 1 * -----	1-15	
			TECHNICAL FIELDS SEARCHED (Int.Cl.7)

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ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 03 01 0184

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
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28-08-2003

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WO 0118170	A	15-03-2001	AU	7323000 A	10-04-2001
			EP	1206559 A2	22-05-2002
			WO	0118170 A2	15-03-2001
US 4282361	A	04-08-1981	NONE		
DE 10060388	A	06-06-2002	DE	10060388 A1	06-06-2002
			AU	1603302 A	18-06-2002
			WO	0245716 A1	13-06-2002

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82